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INVENTORS/APPLICANTS

Given Name (last name, first name, and middle initial [if any])			Residence (City and State or Foreign Country)
Nakamura	Yusuke		Kanagawa, Japan
Nakagawa	Hidewaki		Tokyo, Japan

TITLE OF THE INVENTION

COMPOSITIONS AND METHODS FOR TREATING PANCREATIC CANCER

CORRESPONDENCE ADDRESS

Attorney Name: Firm Name and Address:	Ingrid A. Beattie, Ph.D., J.D. MINTZ, LEVIN, COHN, FERRIS, GLOVSKY AND POPEO, P.C. One Financial Center Boston, MA 02111
Telephone:	(617) 542-6000
Fax:	(617) 542-2241

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Respectfully submitted,

I A Beattie

Date: March 24, 2004

Ingrid A. Beattie, Reg. No. 42,306
Cynthia A. Kozakiewicz, Reg. No. 42,764
MINTZ, LEVIN, COHN, FERRIS, GLOVSKY AND POPEO, P.C.
One Financial Center
Boston, MA 02111
Telephone: (617) 542-6000
Customer No. 30623

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DESCRIPTION

COMPOSITIONS AND METHODS FOR TREATING PANCREATIC CANCER

Technical Field

The present invention relates to the field of biological science, more specifically to the field of cancer research. In particular, the present invention relates a composition comprising a small interfering RNA (siRNA) of PCDH1, CDH3, GPR107 or EphA4.

Background Art

Pancreatic ductal adenocarcinoma (PDACa) is the fifth leading cause of cancer death in the western world and has one of the highest mortality rates of any malignancy, with a 5-year survival rate only 4%. In USA, each year, estimated 30,700 patients are diagnosed with pancreatic cancer and nearly 30,000 will die of these diseases. The vast majority of patients are diagnosed at an advanced stage of disease at which it has no response to current therapies and the patients can survive for few months. Only surgical resection can offer the possibility of cure, but only 10-20% of patients with PDACa can undergo potentially curative resection and even after curative surgery, 80-90% of the patients relapse and die of the disease. Some improvements in surgical outcome or quality of life occur in patients who also receive chemotherapy including gemcitabine and/or radiation, although the impact on long-term survival has been minimal due to the intense resistance of PDACa to any treatment. At this point, management of most patients focuses on palliation.

Therefore, establishment of a novel molecular therapy for PDACa and identification of novel therapeutic molecular targets for PDACa are urgent issues for pancreatic cancer treatment now.

Disclosure of the Invention

The present invention based on the surprising discovery that small interfering RNAs (siRNAs) selective for PCDH1, CDH3, GPR107 or EphA4 are effective for

inhibiting the cellular growth of various cancer cells, including those involved in PDACa. The inventions described in this application are based in part on this discovery.

The invention provides methods for inhibiting cell growth. Among the methods provided are those comprising contacting a cell with a composition comprising a small interfering RNA (siRNA) of PCDH1, CDH3, GPR107 or EphA4. The invention also provides methods for inhibiting tumor cell growth in a subject. Such methods include administering to a subject a composition comprising a small interfering RNA (siRNA) of PCDH1, CDH3, GPR107 or EphA4. Another aspect of the invention provides methods for inhibiting the expression of the PCDH1, CDH3, GPR107 or EphA4 gene in a cell of a biological sample. Expression of the gene may be inhibited by introduction of a double stranded ribonucleic acid (RNA) molecule into the cell in an amount sufficient to inhibit expression of the PCDH1, CDH3, GPR107 or EphA4 gene. Another aspect of the invention relates to products including nucleic acid sequences and vectors as well as to compositions comprising them, useful, for example, in the provided methods. Among the products provided are siRNA molecules having the property to inhibit expression of the PCDH1, CDH3, GPR107 or EphA4 gene when introduced into a cell expressing said gene. Among such molecules are those that comprise a sense strand and an antisense strand, wherein the sense strand comprises a ribonucleotide sequence corresponding to a PCDH1, CDH3, GPR107 or EphA4 target sequence, and wherein the antisense strand comprises a ribonucleotide sequence which is complementary to said sense strand. The sense and the antisense strands of the molecule hybridize to each other to form a double-stranded molecule.

As used herein, the term "organism" refers to any living entity comprised of at least one cell. A living organism can be as simple as, for example, a single eukaryotic cell or as complex as a mammal, including a human being.

As used herein, the term "biological sample" refers to a whole organism or a subset of its tissues, cells or component parts (e.g. body fluids, including but not limited to blood, mucus, lymphatic fluid, synovial fluid, cerebrospinal fluid, saliva, amniotic fluid, amniotic cord blood, urine, vaginal fluid and semen). "Biological sample" further refers to a homogenate, lysate, extract, cell culture or tissue culture prepared from a whole organism or a subset of its cells, tissues or component parts, or a fraction or portion thereof. Lastly, "biological sample" refers to a medium, such as a nutrient broth or gel in which an

organism has been propagated, which contains cellular components, such as proteins or nucleic acid molecules.

The invention features methods of inhibiting cell growth. Cell growth is inhibited by contacting a cell with a composition of a small interfering RNA (siRNA) of PCDH1, CDH3, GPR107 or EphA4. The cell is further contacted with a transfection-enhancing agent. The cell is provided *in vitro, in vivo* or *ex vivo*. The subject is a mammal, *e.g.*, a human, non-human primate, mouse, rat, dog, cat, horse, or cow. The cell is a pancreatic ductal cell. Alternatively, the cell is a tumor cell (*i.e.*, cancer cell) such as a carcinoma cell or an adenocarcinoma cell. For example, the cell is a pancreatic ductal adenocarcinoma cell. By inhibiting cell growth is meant that the treated cell proliferates at a lower rate or has decreased viability than an untreated cell. Cell growth is measured by proliferation assays known in the art.

By the term "siRNA" is meant a double stranded RNA molecule which prevents translation of a target mRNA. Standard techniques of introducing siRNA into the cell are used, including those in which DNA is a template from which RNA is transcribed. The siRNA includes a sense PCDH1, CDH3, GPR107 or EphA4 nucleic acid sequence, an anti-sense PCDH1, CDH3, GPR107 or EphA4 nucleic acid sequence or both. The siRNA is constructed such that a single transcript has both the sense and complementary antisense sequences from the target gene, e.g., a hairpin.

The method is used to alter gene expression in a cell in which expression of PCDH1, CDH3, GPR107 or EphA4 is upregulated, e.g., as a result of malignant transformation of the cells. Binding of the siRNA to an PCDH1, CDH3, GPR107 or EphA4 transcript in the target cell results in a reduction in PCDH1, CDH3, GPR107 or EphA4 production by the cell. The length of the oligonucleotide is at least 10 nucleotides and may be as long as the naturally-occurring PCDH1, CDH3, GPR107 or EphA4 transcript. Preferably, the oligonucleotide is 19-25 nucleotides in length. Most preferably, the oligonucleotide is less than 75, 50 , or 25 nucleotides in length. Examples of siRNA oligonucleotides of PCDH1, CDH3, GPR107 or EphA4 which inhibit PCDH1, CDH3, GPR107 or EphA4 expression in mammalian cells include oligonucleotides containing target sequences, for example, nucleotides of SEQ ID NOs: 54, 57, 60 or 66, respectively.

Methods for designing double stranded RNA having the ability to inhibit gene expression in a target cell are known. (See for example, US Patent No. 6,506,559, herein

incorporated by reference in its entirety). For example, a computer program for designing siRNAs is available from the Ambion website (http://www.ambion.com/techlib/misc/siRNA_finder.html).

The computer program selects nucleotide sequences for siRNA synthesis based on the following protocol.

Selection of siRNA Target Sites

1. Beginning with the AUG start codon of the transcript, scan downstream for AA dinucleotide sequences. Record the occurrence of each AA and the 3' adjacent 19 nucleotides as potential siRNA target sites. Tuschl et al. recommend against designing siRNA to the 5' and 3' untranslated regions (UTRs) and regions near the start codon (within 75bases) as these may be richer in regulatory protein binding sites. UTR-binding proteins and/or translation initiation complexes may interfere with binding of the siRNA endonuclease complex.
2. Compare the potential target sites to the appropriate genome database (human, mouse, rat, etc.) and eliminate from consideration any target sequences with significant homology to other coding sequences. It is suggested to use BLAST, which can be found on the NCBI server at: www.ncbi.nlm.nih.gov/BLAST/
3. Select qualifying target sequences for synthesis. Selecting several target sequences along the length of the gene to evaluate is typical.

Also included in the invention are isolated nucleic acid molecules that include the nucleic acid sequence of target sequences, for example, nucleotides of SEQ ID NOs: 54, 57, 60 and 66 or a nucleic acid molecule that is complementary to the nucleic acid sequence of nucleotides of SEQ ID NOs: 54, 57, 60 and 66. As used herein, an "isolated nucleic acid" is a nucleic acid removed from its original environment (e.g., the natural environment if naturally occurring) and thus, synthetically altered from its natural state. In the present invention, isolated nucleic acid includes DNA, RNA, and derivatives thereof. When the isolated nucleic acid is RNA or derivatives thereof, base "t" shoulde be replaced with "u" in the nucleotide sequences. As used herein, the term "complementary" refers to Watson-Crick or Hoogsteen base pairing between nucleotides units of a nucleic acid molecule, and the term "binding" means the physical or chemical interaction between two polypeptides or compounds or associated polypeptides or compounds or combinations

thereof. Complementary nucleic acid sequences hybridize under appropriate conditions to form stable duplexes containing few or no mismatches. Furthermore, the sense strand and antisense strand of the isolated nucleotide of the present invention, can form double stranded nucleotide or hairpin loop structure by the hybridization. In a preferred embodiment, such duplexes contain no more than 1 mismatch for every 10 matches. In an especially preferred embodiment, where the strands of the duplex are fully complementary, such duplexes contain no mismatches. The nucleic acid molecule is less than 3581, 3205, 6840 or 3468 nucleotides in length for PCDH1, CDH3, GPR107 or EphA4, respectively. For example, the nucleic acid molecule is less than 500, 200, or 75 nucleotides in length. Also included in the invention is a vector containing one or more of the nucleic acids described herein, and a cell containing the vectors. The isolated nucleic acids of the present invention are useful for siRNA against PCDH1, CDH3, GPR107 or EphA4, or DNA encoding the siRNA. When the nucleic acids are used for siRNA or coding DNA thereof, the sense strand is preferably longer than 19 nucleotides, and more preferably longer than 21 nucleotides.

The invention is based in part on the discovery that the gene encoding PCDH1, CDH3, GPR107 or EphA4 is overexpressed in pancreatic ductal adenocarcinoma (PDACa) compared to non-cancerous pancreatic tissue. The cDNA of PCDH1, CDH3, GPR107 or EphA4 is 3581, 3205, 6840 or 3468 nucleotides in length. The nucleic acid and polypeptide sequences of PCDH1, CDH3, GPR107 or EphA4 are shown in SEQ ID NO: 1 and 2, 3 and 4, 5 and 6 or 7 and 8, respectively. The sequence data are also available via following accession numbers.

PCDH1(CFUPC): L11370, NM_002587

CDH3: X63629, AB046844

GPR107: NM_032925, (KIAA1624: R39794)

EphA4: L36645, NM_004438

Transfection of siRNAs comprising SEQ ID NOs: 54, 57, 60 and 66 resulted in a growth inhibition of PDACa cell lines. PCDH1 (CFUPC) belongs to the protocadherin family, the largest subgroup of cadherin superfamily of calcium-dependent cell-cell adhesion molecules. Many of the protocadherin are highly expressed in the central nervous system and they are likely to play roles in neuronal circuit development and the modulation of synaptic transmission (Sano K, Tanihara H, Heimark RL, Obata S,

Davidson M, St John T, Taketani S, Suzuki S. Protocadherins: a large family of cadherin-related molecules in central nervous system. *EMBO J.*, 12:2249-56, 1993.Frank M, and Kemler R. Protocadherins. *Curr Opin Cell Biol.*, 14:557-62, 2002). However, PCDH1 is abundant in pancreatic cancer cells, but not in central nervous system (Figure 3A), and its function remains unknown.

CDH3 is also a classical member of the cadherin family (Shimoyama Y, Yoshida T, Terada M, Shimosato Y, Abe O, Hirohashi S. Molecular cloning of a human Ca2+-dependent cell-cell adhesion molecule homologous to mouse placental cadherin: its low expression in human placental tissues. *J Cell Biol.*, 109:1787-94. 1989) and they link to catenins and cytoskeletons through its conserved intracellular domain, mediating signal-transduction that control cell polarity, differentiation, motility and cell growth (Christofori G. Changing neighbours, changing behaibour: cell adhesion molecules-mediated signaling during tumor progression. *EMBO J.*, 22, 2318-2323, 2003). However, different form E-cadherin or N-cadherin, the function of CDH3 still remains unclear. Its expression is observed in mammary glands and ovary, and loss of expression was reported in breast cancer and prostate cancer, although the expression of P-cadherin in breast cancer correlates with poor prognosis (Peralta Soler A, Knudsen KA, Salazar H, Han AC, Keshgegian AA. P-cadherin expression in breast carcinoma indicates poor survival. *Cancer*, 86:1263-1272. 1999).

GPR107 (KIAA1624) is one of the G protein-coupled receptors (GPCR) with seven transmembranes. A large percentage of today's prescription drugs target one or more GPCRs with most major therapeutic area being served to some extent by several GPCR-based drugs. Clearly, GPCRs are in the highest rank in the terms of drug discovery potential. GPR107 is expressed inrestrictedly in normal heart, placenta, skeletal muscle, prostate, testis, ovary, spinal cord as shown in Northern blot analysis (Figure 3C). This is not abundant in major vital organs, suggesting that targeting for these molecules would be expected to lead less toxicity in human body.

EphA4 is one of the receptor with tyrosine kinase activity and their functions with their ephrin ligands are best studies in the nervous system, where Eph receptors and ephrin molecules are involved in patterning the developing hindbrain, axon pathfinding and guiding neural crest cell migration (Dodelet VC, and Pasquale EB. Eph receptors and ephrin lignads: embryogenesis to tumorigenesis. *Oncogene*, 19: 5614-5619, 2000). These

molecules also regulate embryonic vascular development and there are some reports about the association of Eph/ephrin with tumor angiogenesis (Dodelet VC, and Pasquale EB. Eph receptors and ephrin lignads: embryogenesis to tumorigenesis. *Oncogene*, 19: 5614-5619, 2000). The Eph receptor family consists of 13 members and their ligands, ephrins, are divided into two subclasses, the A-subclass (A1-A5) and the B-subclass (B1-B3). The receptors are divided on the basis of sequence similarity and ligand affinity into A–subclass (EphA1-A8), and B-subclass (EphB1-B4, B6). A-type receptors typically bind to most or all A-type ligands, and B-type receptors bind to most or all B-type ligands, with the exception of EphA4 that can bind both A-type and most B-type ligands (Dodelet VC, and Pasquale EB. Eph receptors and ephrin lignads: embryogenesis to tumorigenesis. *Oncogene*, 19: 5614-5619, 2000).

<u>Methods of inhibiting cell growth</u>

The present invention relates to inhibiting cell growth, i.e, cancer cell growth by inhibiting expression of PCDH1, CDH3, GPR107 or EphA4. Expression of PCDH1, CDH3, GPR107 or EphA4 is inhibited by small interfering RNA (siRNA) that specifically target the PCDH1, CDH3, GPR107 or EphA4 gene. PCDH1, CDH3, GPR107 or EphA4 targets include, for example, nucleotides of SEQ ID NOs: 54, 57, 60 and 66.

In non-mammalian cells, double-stranded RNA (dsRNA) has been shown to exert a strong and specific silencing effect on gene expression, which is referred as RNA interference (RNAi) (1). dsRNA is processed into 20-23 nucleotides dsRNA called small interfering RNA (siRNA) by an enzyme containing RNase III motif. The siRNA specifically targets complementary mRNA with a multicomponent nuclease complex (2, 3). In mammalian cells, siRNA composed of 20 or 21-mer dsRNA with 19 complementary nucleotides and 3' terminal noncomplementary dimmers of thymidine or uridine, have been shown to have a gene specific knock-down effect without inducing global changes in gene expression (4). In addition, plasmids containing small nuclear RNA (snRNA) U6 or polymerase III H1-RNA promoter effectively produce such short RNA recruiting type III class of RNA polymerase III and thus can constitutively suppress its target mRNA (5, 6).

The growth of cells are inhibited by contacting a cell, with a composition containing a siRNA of PCDH1, CDH3, GPR107 or EphA4. The cell is further contacted with a transfection agent. Suitable transfection agents are known in the art. By inhibition

of cell growth is meant the cell proliferates at a lower rate or has decreased viability compared to a cell not exposed to the composition. Cell growth is measured by methods known in the art such as, the MTT cell proliferation assay.

The siRNA of PCDH1, CDH3, GPR107 or EphA4 is directed to a single target of PCDH1, CDH3, GPR107 or EphA4 gene sequence. Alternatively, the siRNA is directed to multiple target of PCDH1, CDH3, GPR107 or EphA4 gene sequences. For example, the composition contains siRNA of PCDH1, CDH3, GPR107 or EphA4 directed to two, three, four, or five or more target sequences of PCDH1, CDH3, GPR107 or EphA4. By PCDH1, CDH3, GPR107 or EphA4 target sequence is meant a nucleotide sequence that is identical to a portion of the PCDH1, CDH3, GPR107 or EphA4 gene. The target sequence can include the 5' untranslated (UT) region, the open reading frame (ORF) or the 3' untranslated region of the human PCDH1, CDH3, GPR107 or EphA4 gene. Alternatively, the siRNA is a nucleic acid sequence complementary to an upstream or downstream modulator of PCDH1, CDH3, GPR107 or EphA4 gene expression. Examples of upstream and downstream modulators include, a transcription factor that binds the PCDH1, CDH3, GPR107 or EphA4 gene promoter, a kinase or phosphatase that interacts with the PCDH1, CDH3, GPR107 or EphA4 polypeptide, a PCDH1, CDH3, GPR107 or EphA4 promoter or enhancer.

siRNA of PCDH1, CDH3, GPR107 or EphA4 which hybridize to target mRNA decrease or inhibit production of the PCDH1, CDH3, GPR107 or EphA4 polypeptide product encoded by the PCDH1, CDH3, GPR107 or EphA4 gene by associating with the normally single-stranded mRNA transcript, thereby interfering with translation and thus, expression of the protein. The siRNA is less than 500, 200, 100, 50, or 25 nucleotides in length. Preferably the siRNA is 19-25 nucleotides in length. Exemplary nucleic acid sequence for the production of PCDH1, CDH3, GPR107 or EphA4 siRNA include the sequences of nucleotides of SEQ ID NOs: 54, 57, 60 or 66 as the target sequence, respectively. Furthermore, in order to enhance the inhibition activity of the siRNA, nucleotide "u" can be added to 3'end of the antisense strand of the target sequence. The number of "u"s to be added is at least 2, generally 2 to 10, preferably 2 to 5. The added "u"s form single strand at the 3'end of the antisense strand of the siRNA.

The cell is any cell that expresses or over-expresses PCDH1, CDH3, GPR107 or EphA4. The cell is an epithelial cell such as a pancreatic ductal cell. Alternatively, the

cell is a tumor cell such as a carcinoma, adenocarcinoma, blastoma, leukemia, myeloma, or sarcoma. The cell is a pancreatic ductal adenocarcinoma.

An siRNA of PCDH1, CDH3, GPR107 or EphA4 is directly introduced into the cells in a form that is capable of binding to the mRNA transcripts. Alternatively, the DNA encoding the siRNA of PCDH1, CDH3, GPR107 or EphA4 is in a vector.

Vectors are produced for example by cloning a PCDH1, CDH3, GPR107 or EphA4 target sequence into an expression vector operatively-linked regulatory sequences flanking the PCDH1, CDH3, GPR107 or EphA4 sequence in a manner that allows for expression (by transcription of the DNA molecule) of both strands (Lee, N.S., Dohjima, T., Bauer, G., Li, H., Li, M.-J., Ehsani, A.,Salvaterra, P., and Rossi, J. (2002) Expression of small interfering RNAs targeted against HIV-1 rev transcripts in human cells. Nature Biotechnology 20 : 500-505.). An RNA molecule that is antisense to PCDH1, CDH3, GPR107 or EphA4 mRNA is transcribed by a first promoter (e.g., a promoter sequence 3' of the cloned DNA) and an RNA molecule that is the sense strand for the PCDH1, CDH3, GPR107 or EphA4 mRNA is transcribed by a second promoter (e.g., a promoter sequence 5' of the cloned DNA). The sense and antisense strands hybridize *in vivo* to generate siRNA constructs for silencing of the PCDH1, CDH3, GPR107 or EphA4 gene. Alternatively, two constructs are utilized to create the sense and anti-sense strands of a siRNA construct. Cloned PCDH1, CDH3, GPR107 or EphA4 can encode a construct having secondary structure, e.g., hairpins, wherein a single transcript has both the sense and complementary antisense sequences from the target gene.

A loop sequence consisting of an arbitrary nucleotide sequence can be located between the sense and antisense sequence in order to form the hairpin loop structure. Thus, the present invention also provides siRNA having the general formula 5'-[A]-[B]-[A']-3', wherein [A] is a ribonucleotide sequence corresponding to a sequence selected from the group consisting of nucleotides of SEQ ID NOs: 54, 57, 60 and 66,

[B] is a ribonucleotide sequence consisting of 3 to 23 nucleotides, and

[A'] is a ribonucleotide sequence consisting of the complementary sequence of [A]

The region [A] hybridizes to [A'], and then a loop consisting of region [B] is formed. The loop sequence may be preferably 3 to 23 nucleotide in length. The loop sequence, for example, can be selected from group consisting of following sequences (http://www.ambion.com/techlib/tb/tb_506.html). Furthermore, loop sequence consisting

of 23 nucleotides also provides active siRNA (Jacque, J.-M., Triques, K., and Stevenson, M. (2002) Modulation of HIV-1 replication by RNA interference. Nature 418 : 435-438.).

CCC, CCACC or CCACACC: Jacque, J. M., Triques, K., and Stevenson, M (2002) Modulation of HIV-1 replication by RNA interference. Nature, Vol. 418: 435-438.

UUCG: Lee, N.S., Dohjima, T., Bauer, G., Li, H., Li, M.-J., Ehsani, A., Salvaterra, P., and Rossi, J. (2002) Expression of small interfering RNAs targeted against HIV-1 rev transcripts in human cells. Nature Biotechnology 20 : 500-505. Fruscoloni, P., Zamboni, M., and Tocchini-Valentini, G. P. (2003) Exonucleolytic degradation of double-stranded RNA by an activity in Xenopus laevis germinal vesicles. Proc. Natl. Acad. Sci. USA 100(4): 1639-1644..

UUCAAGAGA: Dykxhoorn, D. M., Novina, C. D., and Sharp, P. A. (2002) Killing the messenger: Short RNAs that silence gene expression. Nature Reviews Molecular Cell Biology 4: 457-467.

For example, preferable siRNAs having hairpin loop structure of the present invention are shown below. In the following structure, the loop sequence can be selected from group consisting of CCC, UUCG, CCACC, CCACACC, and UUCAAGAGA. Preferable loop sequence is UUCAAGAGA ("ttcaagaga" in DNA).

GACAUCAAUGACAACACAC-[B]-GUGUGUUGUCAUUGAUGUC (for target sequence of SEQ ID NO:54)

GGAGACAGGCUGGUUGUUG-[B]-CAACAACCAGCCUGUCUCC (for target sequence of SEQ ID NO:57)

GUGGCUCUACCAGCUCCUG-[B]-CAGGAGCUGGUAGAGCCAC (for target sequence of SEQ ID NO:60)

GCAGCACCAUCAUCCAUUG-[B]-CAAUGGAUGAUGGUGCUGC (for target sequence of SEQ ID NO:66)

The regulatory sequences flanking the PCDH1, CDH3, GPR107 or EphA4 sequence are identical or are different, such that their expression can be modulated independently, or in a temporal or spatial manner. siRNAs are transcribed intracellularly

by cloning the PCDH1, CDH3, GPR107 or EphA4 gene templates into a vector containing, e.g., a RNA pol III transcription unit from the small nuclear RNA (snRNA) U6 or the human H1 RNA promoter. For introducing the vector into the cell, transfection-enhancing agent can be used. FuGENE (Rochediagnostices), Lipofectamin 2000 (Invitrogen), Oligofectamin (Invitrogen), and Nucleofactor (Wako pure Chemical) are useful as the transfection-enhancing agent.

Oligonucleotides and oligonucleotides complementary to various portions of PCDH1, CDH3, GPR107 or EphA4 mRNA were tested *in vitro* for their ability to decrease production of PCDH1, CDH3, GPR107 or EphA4 in tumor cells (e.g., using the pancreatic cell line such as pancreatic ductal adenocarcinoma(PDACa) cell line) according to standard methods. A reduction in PCDH1, CDH3, GPR107 or EphA4 gene product in cells contacted with the candidate siRNA composition compared to cells cultured in the absence of the candidate composition is detected using specific antibodies of PCDH1, CDH3, GPR107 or EphA4 or other detection strategies. Sequences which decrease production of PCDH1, CDH3, GPR107 or EphA4 in *in vitro* cell-based or cell-free assays are then tested for there inhibitory effects on cell growth. Sequences which inhibit cell growth *in vitro* cell-based assay are test *in vivo* in rats or mice to confirm decreased PCDH1, CDH3, GPR107 or EphA4 production and decreased tumor cell growth in animals with malignant neoplasms.

<u>Methods of treating malignant tumors</u>

Patients with tumors characterized as over-expressing PCDH1, CDH3, GPR107 or EphA4 are treated by administering siRNA of PCDH1, CDH3, GPR107 or EphA4. siRNA therapy is used to inhibit expression of PCDH1, CDH3, GPR107 or EphA4 in patients suffering from or at risk of developing, for example, pancreatic ductal adenocarcinoma (PDACa). Such patients are identified by standard methods of the particular tumor type. Pancreatic ductal adenocarcinoma (PDACa) is diagnosed for example, by CT, MRI, ERCP, MRCP, computer tomography, or ultrasound. Treatment is efficacious if the treatment leads to clinical benefit such as, a reduction in expression of PCDH1, CDH3, GPR107 or EphA4, or a decrease in size, prevalence, or metastatic potential of the tumor in the subject. When treatment is applied prophylactically, "efficacious" means that the treatment retards or prevents tumors from forming or prevents or alleviates a symptom of clinical symptom.

of the tumor. Efficaciousness is determined in association with any known method for diagnosing or treating the particular tumor type.

siRNA therapy is carried out by administering to a patient a siRNA by standard vectors and/or gene delivery systems. Suitable gene delivery systems may include liposomes, receptor-mediated delivery systems, or viral vectors such as herpes viruses, retroviruses, adenoviruses and adeno-associated viruses, among others. A therapeutic nucleic acid composition is formulated in a pharmaceutically acceptable carrier. The therapeutic composition may also include a gene delivery system as described above. Pharmaceutically acceptable carriers are biologically compatible vehicles which are suitable for administration to an animal, e.g., physiological saline. A therapeutically effective amount of a compound is an amount which is capable of producing a medically desirable result such as reduced production of a PCDH1, CDH3, GPR107 or EphA4 gene product, reduction of cell growth, e.g., proliferation, or a reduction in tumor growth in a treated animal.

Parenteral administration, such as intravenous, subcutaneous, intramuscular, and intraperitoneal delivery routes, may be used to deliver siRNA compositions of PCDH1, CDH3, GPR107 or EphA4. For treatment of pancreatic tumors, direct infusion the celiac artery, splenic artery, or common hepatic artery, is useful.

Dosages for any one patient depends upon many factors, including the patient's size, body surface area, age, the particular nucleic acid to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. Dosage for intravenous administration of nucleic acids is from approximately 10^6 to 10^{22} copies of the nucleic acid molecule.

The polynucleotides are administered by standard methods, such as by injection into the interstitial space of tissues such as muscles or skin, introduction into the circulation or into body cavities or by inhalation or insufflation. Polynucleotides are injected or otherwise delivered to the animal with a pharmaceutically acceptable liquid carrier, e.g., a liquid carrier, which is aqueous or partly aqueous. The polynucleotides are associated with a liposome (e.g., a cationic or anionic liposome). The polynucleotide includes genetic information necessary for expression by a target cell, such as a promoters.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this

invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Brief Description of the Drawings

Figure 1 depicts photographs showing the results of validation of over expression of PCDH1 (A) and CDH3 (B) in the PDACa cells by RT-PCR. The microdissected normal pancreatic ductal epithelial cells (Normal) and vital organs (lung, heart, liver, kidney and bone marrow) form the same individual were compared by semiquantitative RT-PCR.

Figure 2 depicts photographs showing the result of immunohistochemistry in PDACa tissues. Overexpression of CDH3 and EphA4 protein was observed in pancreatic ductal adenocarcinoma, but not in normal pancreatic duct.

Figure 3 depicts photographs of Northern blot analysis showing the expression pattern in normal adult tissues of each target genes for pancreatic cancer. (A) PCDH1, (B) CDH3, (C) GPR107, and (D) EphA4.

Figure 4 depicts photographs showing the effect of Knocking-down endogenous PCDH1 in PDACa cell, PK-45P, by siRNA. Figure 4 (A) shows the results of RT-PCR. It validated knockdown effect of PCDH1 mRNA by transfection of siRNA expression vectors 410si, but not by 3344si, 3498si and EGFPsi. The 410si, 3344si, and 3498si were designed specifically for PCDH mRNA sequence, and EGFP was for EGFP mRNA sequence. RNA was harvested 48 hours after transfection and analyzed. ACTB was used to normalize input cDNA. Figure 4 (B) is a photograph showing the results of Colony formation assay. It showed drastic decrease of colony numbers in the cells one week after transfection with 410si that was validated to knock down PCDH1 effectively by RT-PCR. Figure 4 (C) is a photograph showing the results MTT assay. It also showed drastic decreased number of the grown cells transfected with 410si but not by 3344si, 3498si and EGFPsi.

Figure 5 depicts photographs showing the effect of Knocking-down endogenous CDH3 in PDACa cell, KLM-1, by siRNA. Figure 5 (A) shows the results of RT-PCR. It validated knockdown effect of CDH3 mRNA by transfection of siRNA expression vectors si24 but not by si29, si70 and EGFPsi. The si24, si29, and si70 were designed specifically for CDH3 mRNA sequence, and EGFPsi was for EGFP mRNA sequence. RNA was harvested 48 hours after transfection and analyzed. ACTB was used to normalize input cDNA. Figure 5 (B) is a photograph showing the results of Colony formation assay. It showed drastic decrease of colony numbers in the cells one week after transfection with si24 that was validated to knock down CDH3 effectively by RT-PCR. Figure 5 (C) is a photograph showing the results MTT assay. It also showed drastic decreased number of the grown cells transfected with si24, but not by si29, si70 and EGFPsi.

Figure 6 depicts photographs showing the effect of Knocking-down endogenous GPR107 in PDACa cell, KLM-1, by siRNA. Figure 6 (A) shows the results of RT-PCR. It validated knockdown effect of GPR107 mRNA by transfection of siRNA expression vectors 1003si, but not by 1066si, 1118si, 1171si and EGFPsi. The 1003si, 1066si, 1118si, and 1171si were designed specifically for GPR107 mRNA sequence, and EGFPsi was for EGFP mRNA sequence. RNA was harvested 48 hours after transfection and analyzed. ACTB was used to normalize input cDNA. Figure 6 (B) is a photograph showing the results of Colony formation assay. It showed decrease of colony numbers in the cells one week after transfection with 1003si that was validated to knock down GPR107 effectively by RT-PCR. Figure 6 (C) is a photograph showing the results MTT assay. It also showed decreased number of the grown cells transfected with 1003si, but not by1118si, 1171si and EGFPsi.

Figure 7 depicts photographs showing the effect of Knocking-down endogenous EphA4 in PDACa cell, MIA-Paca2, by siRNA. Figure 7 (A) shows the results of RT-PCR. It validated knockdown effect of EphA4 mRNA by transfection of siRNA expression vectors 1313si, but not by 198si, 468si and EGFPsi. The 198si, 468si, 1313si were designed specifically for EphA4 mRNA sequence, and EGFPsi was for EGFP mRNA sequence. RNA was harvested 48 hours after transfection and analyzed. ACTB was used to normalize

input cDNA. Figure 7 (B) is a photograph showing the results of Colony formation assay. It showed drastic decrease of colony numbers in the cells one week after transfection with 1313si that was validated to knock down EphA4 effectively by RT-PCR. Figure 7 (C) is a photograph showing the results MTT assay. It also showed drastic decreased number of the grown cells transfected with1313si, but not by 198si, 468si and EGFPsi.

Best Mode for Carrying out the Invention

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

[Example 1] General Methods

Cell lines and tissue specimens

Human Pancreatic cell lines PK45P, KLM1 and MIA-PaCa2 (ATCC Number: CRL-1420) were obtained from the Cell Resource Center for Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University. All these cells are publicly available.

Isolation of over-expressing genes in PDACa cells by using cDNA microarray

Fabrication of the cDNA microarray slides has been described (Ono K, Tanaka T, Tsunoda T, Kitahara O, Kihara C, Okamoto A, Ochiai K, Takagi T, and Nakamura Y. *Cancer Res.*, 60: 5007-5011, 2000). For each analysis of expression profiles it was prepared duplicate sets of cDNA microarray slides containing approximately 27,000 DNA spots, to reduce experimental fluctuation. Briefly, total RNA was purified from PDACa cells and normal pancreatic duct epithelium microdissected from 18 pancreatic cancer tissues. T7-based RNA amplification was carried out to obtain adequate RNA for microarray experiments. Aliquots of amplified RNA from PDACa cells and normal duct epithelium were labeled by reverse transcription with Cy5-dCTP and Cy3-dCTP, respectively (Amersham Biosciences). Hybridization, washing, and detection were carried out as described previously (Ono K, Tanaka T, Tsunoda T, Kitahara O, Kihara C, Okamoto A, Ochiai K, Takagi T, and Nakamura Y. *Cancer Res.*, 60: 5007-5011, 2000). Subsequently, among the up-regulated genes, it was focused four genes, PCDH1, CDH3, GPR107 and EphA4 because its expression ratio was greater than 5.0 in more than 50% of

informative cancers and their expression level in normal vital major organs was relatively low according to the our previous data of gene expression in 29 normal human tissues (Saito-Hisaminato A, Katagiri T, Kakiuchi S, Nakamura T, Tsunoda T, Nakamura Y. Genome-wide profiling of gene expression in 29 normal human tissues with a cDNA microarray. *DNA Res.*, 9: 35-45, 2002).

Semiquantitative RT-PCR for PCDH1, CDH3, GPR107 and EphA4

RNA from the microdissected PDACa cells and normal pancreatic ductal epithelial cells were subject to two-round amplification by T7-based *in vitro* transcription (Epicentre Technologies) and synthesized to single-strand cDNA. It was prepared appropriate dilutions of each single-stranded cDNA for subsequent PCR amplification by monitoring β-actin (ACTB) as a quantitative control. The primer sequences the present inventors used were 5'-AGAAGGAGACCAAGGACCTGTAT-3' (SEQ.ID.NO.9) and 5'-AGAACTTTATTGTCAGGGTCAAGG-3' (SEQ.ID.NO.10) for PCDH1, 5'-CTGAAGGCGGCTAACACAGAC-3' (SEQ.ID.NO.11) and 5'-TACACGATTGTCCTCACCCTTC-3' (SEQ.ID.NO.12) for CDH3, and 5'-CATCCACGAAACTACCTTCAACT-3' (SEQ.ID.NO.13) and 5'-TCTCCTTAGAGAGAAGTGGGGTG-3' (SEQ.ID.NO.14) for ACTB. All reactions involved initial denaturation at 94°C for 2 min followed by 21 cycles (for ACTB) or 28-32 cycles (for PCDH1 and CDH3) at 94°C for 30 s, 58°C for 30 s, and 72°C for 1 min, on a GeneAmp PCR system 9700 (PE Applied Biosystems).

Immunohistochemistry

Formalin-fixed and paraffin-embedded PDACa sections were immunostained using a mouse anti-CDH3 monoclonal antibody (BD Transduction Laboratories) or a rabbit anti-EPHA4 (EphA4) polyclonal antibody (Santa Cruz Biotechnology) for CDH3 and EPHA4 expression. Deparaffinized tissue sections were placed in 10 mM citrate buffer, pH 6.0, and heated to 108°C in an autoclave for 15 minutes for antigen retrieval. Sections were incubated with a 1:10 dilution or a 1:100 dilution of primary antibody for CDH3 or EPHA4, respectively, in a humidity chamber for an hour at room temperature, and developed with peroxidase labeled-dextran polymer followed by diaminobenzidine

(DAKO Envision Plus System; DAKO Corporation, Carpinteria, CA). Sections were counterstained with hematoxylin. For negative controls, primary antibody was omitted.

Northern blot analysis

Human multiple-tissue Northern blots (Clontech) were hybridized with a [α-^{32}P] dCTP-labeled PCR product amplified by the primers described above. Pre-hybridization, hybridization and washing were performed according to the supplier's recommendations. The blots were auto-radiographed with intensifying screens at -80°C for 5 days.

Construction of psiU6BX Plasmid

The DNA flagment encoding siRNA was inserted into the GAP at nucleotide 485-490 as indicated (-) in the following plasmid sequence (SEQ ID No: 67).

```
GACGGATCGGGAGATCTCCCGATCCCCTATGGTGCACTCTCAGTACAATCTGCTCTGGAT
CCACTAGTAACGGCCGCCAGTGTGCTGGAATTCGGCTTGGGGATCAGCGTTTGAGTAAGA
GCCCGCGTCTGAACCCTCCGCGCCGCCCCGGCCCCAGTGGAAAGACGCGCAGGCAAAACG
CACCACGTGACGGAGCGTGACCGCGCGCCGAGCGCGCGCCAAGGTCGGGCAGGAAGAGGG
CCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAAT
TAGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTA
ATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCT
TACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAA
CACC------TTTTTACATCAGGTTGTTTTTCTGTTTGGTTTTTTTTTTACACCACGTTT
ATACGCCGGTGCACGGTTTACCACTGAAAACACCTTTCATCTACAGGTGATATCTTTTAA
CACAAATAAAATGTAGTAGTCCTAGGAGACGGAATAGAAGGAGGTGGGGCCTAAAGCCGA
ATTCTGCAGATATCCATCACACTGGCGGCCGCTCGAGTGAGGCGGAAAGAACCAGCTGGG
GCTCTAGGGGGTATCCCCACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGG
TTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCT
TCCCTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCC
CTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAAACTTGATTAGGGTG
ATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGT
CCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGG
TCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTCGGCCTATTGGTTAAAAAATGAGC
TGATTTAACAAAAATTTAACGCGAATTAATTCTGTGGAATGTGTGTCAGTTAGGGTGTGG
AAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGC
AACCAGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCT
CAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCC
CAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGA
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GGCCGCCTCTGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGG
CTTTTGCAAAAAGCTCCCGGGAGCTTGTATATCCATTTTCGGATCTGATCAAGAGACAGG
ATGAGGATCGTTTCGCATGATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTG
GGTGGAGAGGCTATTCGGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGC
CGTGTTCCGGCTGTCAGCGCAGGGGCGCCCGGTTCTTTTTGTCAAGACCGACCTGTCCGG
TGCCCTGAATGAACTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGT
TCCTTGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGG
CGAAGTGCCGGGGCAGGATCTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCAT
CATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATTCGACCA
CCAAGCGAAACATCGCATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTTGTCGATCA
GGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCGCCAGGCTCAA
GGCGCGCATGCCCGACGGCGAGGATCTCGTCGTGACCCATGGCGATGCCTGCTTGCCGAA
TATCATGGTGGAAAATGGCCGCTTTTCTGGATTCATCGACTGTGGCCGGCTGGGTGTGGC
GGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGCGA
ATGGGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCTCCCGATTCGCAGCGCATCGC
CTTCTATCGCCTTCTTGACGAGTTCTTCTGAGCGGGACTCTGGGGTTCGAAATGACCGAC
CAAGCGACGCCCAACCTGCCATCACGAGATTTCGATTCCACCGCCGCCTTCTATGAAAGG
TTGGGCTTCGGAATCGTTTTCCGGGACGCCGGCTGGATGATCCTCCAGCGCGGGGATCTC
ATGCTGGAGTTCTTCGCCCACCCCAACTTGTTTATTGCAGCTTATAATGGTTACAAATAA
AGCAATAGCATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGT
TTGTCCAAACTCATCAATGTATCTTATCATGTCTGTATACCGTCGACCTCTAGCTAGAGC
TTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCA
CACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAA
CTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAG
CTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCC
GCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCT
CACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATG
TGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTC
CATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGA
AACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCT
CCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTG
GCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAG
CTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTAT
CGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAAC
AGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAAC
TACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTC
GGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTTTTTTT
GTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTT
TCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGA

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TTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATC
TAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCT
ATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATA
ACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCA
CGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGA
AGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGA
GTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTG
GTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGA
GTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTT
GTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCT
CTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCA
TTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAAT
ACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGA
AAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCC
AACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGG
CAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTC
CTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTT
GAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCA
CCTGACGTC
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snRNA U6 gene is reported to be transcribed by RNA polymerase III, which produce short transcripts with uridines at the 3' end. The genomic fragment of the snRNA U6 gene containing the promoter region was amplified by PCR using a set of primers,

5'-GGGGATCAGCGTTTGAGTAA-3' (SEQ ID No: 68), and

5'-TAGGCCCCACCTCCTTCTAT-3' (SEQ ID No: 69) and human placental DNA as a template. The product was purified and cloned into pCR plasmid vector using a TA cloning kit according to the supplier's protocol (Invitrogen). The *Bam*HI, *Xho*I fragment containing the snRNA U6 gene was purified and cloned into nucleotide 1257 to 56 fragment of pcDNA3.1(+) plasmid, which was amplified by PCR with a set of primer, 5'-TGCGGATCCAGAGCAGATTGTACTGAGAGT-3' (SEQ ID No: 70) and

5'- CTCTATCTCGAGTGAGGCGGAAAGAACCA-3' (SEQ ID No: 71). The ligated DNA was used for a template of PCR with primers,

5'-TTTAAGCTTGAAGACTATTTTACATCAGGTTGTTTTTCT-3' (SEQ ID No: 72) and

5'-TTTAAGCTTGAAGACACGGTGTTTCGTCCTTTCCACA-3' (SEQ ID No: 73). The product was digested with HindIII, which was subsequently self-ligated to produce psiU6BX vector plasmid. For the control, psiU6BX-EGFP was prepared by cloning double-stranded oligonucleotides of

5'- CACCGAAGCAGCACGACTTCTTCTTCAAGAGAGAAGAAGTCGTGCTGCTTC-3' (SEQ ID No: 74) and

5'- AAAAGAAGCAGCACGACTTCTTCTCTCTTGAAGAAGAAGTCGTGCTGCTTC -3' (SEQ ID No: 75) into the BbsI site in the psiU6BX vector.

siRNA-expressing constructs

The nucleotide sequence of the siRNAs were designed using an siRNA design computer program available from the Ambion website. (http://www.ambion.com/techlib/misc/siRNA_finder.html). Briefly, nucleotide sequences for siRNA synthesis are selected using the following protocol.

Selection of siRNA Target Sites:

1. Starting with the AUG start codon of the each gene transcript, scan downstream for an AA dinucleotide sequences. The occurrence of each AA and the 3' adjacent 19 nucleotides are recorded as potential siRNA target sites. Tuschl et al. recommend against designing siRNA to the 5' and 3' untranslated regions (UTRs) and regions near the start codon (within 75bases) as these may be richer in regulatory protein binding sites. UTR-binding proteins and/or translation initiation complexes may interfere with binding of the siRNA endonuclease complex.

2. The potential target sites are compared to the appropriate genome database (human, mouse, rat, etc.) to eliminate target sequences with significant homology to other coding sequences.

3. Qualifying target sequences are selected for synthesis. Several target sequences along the length of the gene are selected for evaluation.

The oligonucleotides used for siRNAs of PCDH1, CDH3, GPR107 or EphA4 are shown below. Each oligionucleotide is a combination of a sense nucleotide sequence and an antisense nucleotide sequence of the target sequence. The nucleotide sequences of the hairpin loop structure and target sequence are shown in SEQ ID NO:54 to SEQ ID NO:57

and SEQ ID NO:60 to SEQ ID NO:66, respectively (endonuclease recognition cites are eliminated from each hairpin loop structure sequence).

Insert sequence of siRNA for PCDH1

410si:

5'-CACCGACATCAATGACAACACACTTCAAGAGAGTGTGTTGTCATTGATGTC-3' (SEQ ID NO: 15) and

5'-AAAAGACATCAATGACAACACACTCTCTTGAAGTGTGTTGTCATTGATGTC-3' (SEQ ID NO:16)

3344si:

5'-CACCGTCTACTCCAAACCTAGGTTTCAAGAGAACCTAGGTTTGGAGTAGAC-3' (SEQ ID NO: 17) and

5'-AAAAGTCTACTCCAAACCTAGGTTCTCTTGAAACCTAGGTTTGGAGTAGAC-3' (SEQ ID NO: 18)

3498siRNA:

5'-CACCCCTCTTCCTCACCACTAGGTTCAAGAGACCTAGTGGTGAGGAAGAGG-3' (SEQ ID NO: 19) and

5'-AAAACCTCTTCCTCACCACTAGGTCTCTTGAACCTAGTGGTGAGGAAGAGG-3' (SEQ ID NO: 20)

Insert sequence of siRNA for CDH3

si24:

5'-CACCGGAGACAGGCTGGTTGTTGTTCAAGAGACAACAACCAGCCTGTCTCC-3' (SEQ ID NO: 21)and

5'-AAAAGGAGACAGGCTGGTTGTTGTCTCTTGAACAACAACCAGCCTGTCTCC-3' (SEQ ID NO: 22)

si29:

5'-CACCCATCTCCATCATCGTGACCTTCAAGAGAGGTCACGATGATGGAGATG-3' (SEQ ID NO: 23)and

5'-AAAACATCTCCATCATCGTGACCTCTCTTGAAGGTCACGATGATGGAGATG-3' (SEQ ID NO: 24)

si70:

5'-CACCCATCACGGACAAGGACCTGTTCAAGAGACAGGTCCTTGTCCGTGATG-3' (SEQ ID NO: 25)and

5'-AAAACATCACGGACAAGGACCTGTCTCTTGAACAGGTCCTTGTCCGTGATG-3' (SEQ ID NO: 26)

Insert sequence of siRNA for GPR107

1003si:

5'-CACCGTGGCTCTACCAGCTCCTGTTCAAGAGACAGGAGCTGGTAGAGCCAC-3' (SEQ ID NO: 27)and

5'-AAAAGTGGCTCTACCAGCTCCTGTCTCTTGAACAGGAGCTGGTAGAGCCAC-3' (SEQ ID NO: 28)

1066si:

5'-CACCATTCCGTCCGGCTTCAGATTTCAAGAGAATCTGAAGCCGGACGGAAT-3' (SEQ ID NO: 29)and

5'-AAAAATTCCGTCCGGCTTCAGATTCTCTTGAAATCTGAAGCCGGACGGAAT-3' (SEQ ID NO: 30)

1118si:

5'-CACCGACTTGGAAATGGAGTCCGTTCAAGAGACGGACTCCATTTCCAAGTC-3' (SEQ ID NO: 31)and

5'-AAAAGACTTGGAAATGGAGTCCGTCTCTTGAACGGACTCCATTTCCAAGTC-3' (SEQ ID NO: 32)

1171si:

5'-CACCGAAAGTCAAGAAGGTGACCTTCAAGAGAGGTCACCTTCTTGACTTTC-3' (SEQ ID NO: 33) and

5'-AAAAGAAAGTCAAGAAGGTGACCTCTCTTGAAGGTCACCTTCTTGACTTTC-3' (SEQ ID NO: 34)

Insert sequence of siRNA for EphA4

198si:

5'-CACCTCCGAACCTACCAAGTGTGTTCAAGAGACACACTTGGTAGGTTCGGA-3' (SEQ ID NO: 35)and

5'-AAAATCCGAACCTACCAAGTGTGTCTCTTGAACACACTTGGTAGGTTCGGA-3' (SEQ ID NO: 36)

468si:

5'-CACCTCATGAAGCTGAACACCGATTCAAGAGATCGGTGTTCAGCTTCATGA-3' (SEQ ID NO: 37)and

5'-AAAATCATGAAGCTGAACACCGATCTCTTGAATCGGTGTTCAGCTTCATGA-3' (SEQ ID NO: 38)

1313si:

5'-CACCGCAGCACCATCATCCATTGTTCAAGAGACAATGGATGATGGTGCTGC-3' (SEQ ID NO: 39) and

5'-AAAAGCAGCACCATCATCCATTGTCTCTTGAACAATGGATGATGGTGCTGC-3' (SEQ ID NO: 40)

Insert sequence of siRNA for control

EGFPsi: (control)

5'- CACCGAAGCAGCACGACTTCTTCTTCAAGAGAGAAGAAGTCGTGCTGCTTC -3' (SEQ ID NO: 74) and

5'-AAAAGAAGCAGCACGACTTCTTCTCTCTTGAAGAAGAAGTCGTGCTGCTTC-3' (SEQ ID NO: 75)

Sequence ID NO of each sequences are listed in Table1

gene	siRNA	effect	insert seq SEQ ID NO		hairpin siRNA	target SEQ ID NO	position
PCDH1	410si	+	15	16	41	54	595–613
PCDH1	3344si	-	17	18	42	55	3565–3583
PCDH1	3498si	-	19	20	43	56	3719–3737
CDH3	si24	+	21	22	44	57	556–574
CDH3	si29	-	23	24	45	58	670–688
CDH3	si70	-	25	26	46	59	1768–1786
GPR107	1003si	+	27	28	47	60	1570–1588
GPR107	1066si	-	29	30	48	61	1633–1651
GPR107	1118si	-	31	32	49	62	1685–1703
GPR107	1171si	-	33	34	50	63	1738–1756
EphA4	198si	-	35	36	51	64	242–260
EphA4	468si	-	37	38	52	65	530–548
EphA4	1313si	+	39	40	53	66	1357–1375
control	EGFPsi	-	74	75			

colony formation / MTT assay

Human PDACa cell lines among PK45P, KLM1 and MIA-PaCa2, were plated onto 10-cm dishes (5 X 10^5 cells/dish) and transfected with psiU6BX containing EGFP target sequence (EGFP) and psiU6BX containing target sequence using Lipofectamine 2000 (Invitrogen) or FuGENE6 (Roche), according to manufacture's instruction. Cells were selected by 500 mg/ml Geneticin for one week, and preliminary cells were harvested 48 hours after transfection and analyzed by RT-PCR to validate knockdown effect on PCDH1, CDH3, GPR107 and EphA4. The primers of RT-PCR were the same ones described above. These cells were also stained by Giemsa solution and performed MTT assay to evaluate the colony formation and the cell number, respectively.

[Example 2] Reduction of the expression of the genes PCDH1, CDH3, GPR107 or EphA4 and growth suppression of cancer cells by siRNA

In previous study, it was generated precise expression profiles of PDACa by combining laser microdissection with genome-wide cDNA microarrays with 27,000 genes spotted. The present inventors identified more than 200 genes as up-regulated genes in PDACa cells comparing with the expression pattern of normal pancreatic ductal epithelium that was thought to be the origin of PDACa (Nakamura T, Furukawa Y, Nakagawa H,

Tsunoda T, Ohigashi H, Murata K, Ishikawa O, Ohgaki, Kashimura N, Miyamoto M, Hirano S, Kondo S, Katoh H, Nakamura Y, and Katagiri T. Genome-wide cDNA microarray analysis of gene-expression profiles in pancreatic cancers using populations of tumor cells and normal ductal epithelium cells selected for purity by laser microdissection. *Oncogene*, 2004 Feb 9, Epub ahead of print). Based on these expression profile of PDACa cells, the present inventors selected four over-expressing genes, PCDH1 and CDH3 and validated their overexpression in PDACa by RT-PCR using the cDNA from microdissected PDACa cells (Figure 1A,B) or immunohistochemistry (Figure 2). Their products are supposed to be cell–surface membrane proteins that are ideal molecule target for drug design and antibody therapy against cancer. Clinical trials approved that Trastuzumab (Herceptin), a humanized monoclonal antibody against ERBB2 (Her2) is effective for subsets of metastatic breast cancer with HER2 over-expressed, and cell-surface molecules that mediates signaling process necessary for essential cellular functions and for maintaining the malignant phenotypes are now most promising targets for cancer therapy (Pegram M, and Slamon DJ. Biological rationale for Her2/neu as a target for monoclonal antibody therapy. *Semin.Oncology*, 27 (suppl 9): 13-19, 2000). Drug design targeting these membrane molecules can be approached both by blocking their growth-promoting signals and/or by modulating ADCC activity in the same way with Trastuzumab.

(1) PCDH1 (Protocadherin 1) (Genbank Accession No.NM_002587; SEQ ID No.1,2)

To investigate the growth or survival effect of PCDH1 on PDACa cells, the present inventors knocked down their endogenous expression of PCDH1 specifically by mammalian vector-based RNA interference (RNAi) technique in PDACa cell line. PCDH1 is expressed inrestrictedly in normal heart, placenta, prostate as shown in Northern blot analysis (Figure 3A). This is not abundant in major vital organs, suggesting that targeting for these molecules would be expected to lead less toxicity in human body.

The transfection of the siRNA-producing vectors clearly resulted in reduction of the endogenous expression in one designed siRNA, 410si, for PCDH1 (Figure 4A). This knocking-down effect by the siRNA on PCDH1 mRNA resulted in drastic growth suppression in colony formation assay (Figure 4B) and MTT assay (Figure 4C). These findings strongly suggested that overexpression of PCDH1 in PDACa cells were associated with cancer cell viability. PCDH1 and other protocadherins are supported to have homophilic interaction on the cell surface by means of their cadherin domains and

modulate intercellular signal transduction for cytoskeleton conformation, cell motility or cell growth (Sano K, Tanihara H, Heimark RL, Obata S, Davidson M, St John T, Taketani S, Suzuki S. Protocadherins: a large family of cadherin-related molecules in central nervous system. *EMBO J.*, 12:2249-56, 1993, Frank M, and Kemler R. Protocadherins. *Curr Opin Cell Biol.*, 14:557-62, 2002.). According to our data, PCDH1 is likely to modulate positive signal for pancreatic cancer cell growth through its homophilic interaction in cell-cell adhesion.

(2) CDH3 (P-cadherin) (Genbank Accession No.NM_001793; SEQ ID No.3,4)

The present inventors validated CDH3 overexpression in PDACa cells by RT-PCR (Figure 1B) and immunohistochemistry (Figure 2A), and according to the microarray data and RT-PCR (Figure 1B), CDH3 overexpression was one of the most predominant patterns among more than 200 up-regulated genes in our PDACa profiles. CDH3 is expressed inrestrictedly in normal thymus, prostate, ovary, trachea as shown in Northern blot analysis (Figure 3B). This is not abundant in major vital organs, suggesting that targeting for these molecules would be expected to lead less toxicity in human body.

To investigate the growth or survival effect of CDH3 on PDACa cells, the present inventors knocked down their endogenous expression of CDH3 specifically by mammalian vector-based RNA interference (RNAi) technique in PDACa cell line. The transfection of the siRNA-producing vectors clearly resulted in reduction of the endogenous expression in one designed siRNA, si24, for CDH3 (Figure 5A). This knocking-down effect by the siRNA on CDH3 mRNA resulted in drastic growth suppression in colony formation assay (Figure 5B) and MTT assay (Figure 5C). These findings strongly suggested that overexpression of CDH3 in PDACa cells were associated with cancer cell viability as well as cell-cell interaction, and this molecule may involve signal transduction from cell-cell interaction. PDACa is extremely aggressive and high expression of CDH3 in PDACa may be associated with their aggressiveness and metastatic potential as well.

(3) GPR107 (G protein-coupled receptor 107) (Genbank Accession No. AB046844; SEQ ID No.5,6)

The present inventors identified this orphan GPCR as a target for pancreas cancer, which function and ligands are unknown. To investigate the growth or survival effect of GPR107 on PDACa cells, the present inventors knocked down their endogenous expression of GPR107 specifically by siRNA in PDACa cell line. The transfection of the

siRNA-producing vectors clearly resulted in reduction of the endogenous expression in one designed siRNA, 1003si, for GPR107 (Figure 6A). This knocking-down effect by the siRNA on GPR107 mRNA resulted in growth suppression in colony formation assay (Figure 6B) and MTT assay (Figure 6C). These findings strongly suggested that overexpression of GPR107 in PDACa cells were associated with cancer cell viability. Hence, these findings suggested that blocking by antibody or antagonist for GPR107 is a promising approach for PDACa treatment.

(4) EphA4 (Genbank Accession No.NM_004438; SEQ ID No.7,8)

The present inventors validated EphA4 overexpression in PDACa by RT-PCR and immunohistochemistry (Figure 2B), but in pancreatic cancer tissues, the ligand of EphA4 is unknown. Northern blot analysis (Figure 3D) showed that EphA4 was abundant in testis, not in central nervous system and other major organs. Recently the antibody targeting against other Eph receptor family member, EphA2 that is also over-expressed in several cancers, was reported to inhibit breast cancer cell growth *in vitro* and *in vivo* (Carles-Kinch K, Kilpatrick KE, Stewart JC, Kinch MS. Antibody targeting of the EphA2 tyrosine kinase inhibits malignant cell behavior. *Cancer Res.*, 62:2840-2847, 2002). However, EphA2 is expressed ubiquitously in adult tissues, indicating much more possibility of toxicity in treatment of antibody therapy. To investigate the growth or survival effect of EphA4 on PDACa cells, the present inventors knocked down their endogenous expression of EphA4 specifically by siRNA in PDACa cell line. The transfection of the siRNA-producing vectors clearly resulted in reduction of the endogenous expression in one designed siRNA, 1313si, for EphA4 (Figure 7A). This knocking-down effect by the siRNA on EphA4 mRNA resulted in drastic growth suppression in colony formation assay (Figure 7B) and MTT assay (Figure 7C). Considering its tyrosine kinase activity, membrane localization and its specific expression pattern, EphA4 is one the most ideal molecular targets for pancreatic cancer.

In conclusion, the present inventors identified four membrane-type molecules over-expressed in PDACa cells and all of them are likely to be associated with cancer cell growth, suggested these membrane-type molecules are ideal molecular targets for deadly pancreatic cancer treatment and antibodies against these membrane molecules are promising therapeutic approach.

Industrial Applicability

The present inventors have shown that the cell growth is suppressed by small interfering RNA (siRNA) that specifically target the PCDH1, CDH3, GPR107 or EphA4 gene. Thus, this novel siRNAs are useful target for the development of anti-cancer pharmaceuticals. For example, agents that block the expression of PCDH1, CDH3, GPR107 or EphA4 or prevent its activity may find therapeutic utility as anti-cancer agents, particularly anti-cancer agents for the treatment of pancreatic cancer, such as pancreatic ductal adenocarcinoma (PDACa).

While the invention has been described in detail and with reference to specific embodiments thereof, it will be apparent to one skilled in the art that various changes and modifications can be made therein without departing from the spirit and scope of the invention.

References

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(2) Hammond SM, Bernstein E, Beach D, Hannon GJ. An RNA-directed nuclease mediates post-transcriptional gene silencing in Drosophila cells. Nature. 2000 Mar 16;404(6775):293-6.

(3) Hannon GJ. RNA interference. Nature. 2002 Jul 11;418(6894):244-51.

(4) Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature. 2001 May 24;411(6836):494-8.

(5) Miyagishi M, Taira K. U6 promoter-driven siRNAs with four uridine 3' overhangs efficiently suppress targeted gene expression in mammalian cells.Nat Biotechnol. 2002 May;20(5):497-500

(6) Brummelkamp TR, Bernards R, Agami R. A System for Stable Expression of Short Interfering RNAs in Mammalian Cells Science. 296(5567):550-553, April 19, 2002.

CLAIMS

1. A method for treating or preventing pancreatic cancer in a subject comprising administering to said subject a composition comprising a small interfering RNA (siRNA) of *PCDH1*, *CDH3*, *GPR107* or *EPHA4*.

2. The method of claim 1, wherein said siRNA comprises a sense nucleic acid and an anti-sense nucleic acid of *PCDH1*, *CDH3*, *GPR107* or *EPHA4*.

3. The method of claim 1, wherein the pancreatic cancer is an pancreatic ductal adenocarcinoma (PDACa).

4. The method of claim 2, wherein the siRNA comprises a ribonucleotide sequence corresponding to a sequence selected from the group consisting of SEQ ID NOs: 54, 57, 60 and 66 as the target sequence.

5. The method of claim 4, said siRNA has the general formula 5'-[A]-[B]-[A']-3', wherein [A] is a ribonucleotide sequence corresponding to a sequence selected from the group consisting of nucleotides of SEQ ID NOs: 54, 57, 60 and 66.
[B] is a ribonucleotide sequence consisting of 3 to 23 nucleotides, and
[A'] is a ribonucleotide sequence consisting of the complementary sequence of [A].

6. The method of claim 1, wherein said composition comprises a transfection-enhancing agent.

7. A double-stranded molecule comprising a sense strand and an antisense strand, wherein the sense strand comprises a ribonucleotide sequence corresponding to a target sequence selected from the group consisting of SEQ ID NOs: 54, 57, 60 and 66, and wherein the antisense strand comprises a ribonucleotide sequence which is complementary to said sense strand, wherein said sense strand and said antisense strand hybridize to each other to form said double-stranded molecule, and wherein said double-stranded molecule, when introduced into a cell expressing the *PCDH1*, *CDH3*, *GPR107* or *EPHA4* gene, inhibits expression of said gene.

8. The double-stranded molecule of claim 7, wherein said target sequence comprises at least about 10 contiguous nucleotides from the nucleotide sequences selected from the group of SEQ ID NOs: 1, 3, 5, and 7.

9. The double-stranded molecule of claim 8, wherein said target sequence comprises from about 19 to about 25 contiguous nucleotides from the nucleotide sequences selected from the group of SEQ ID NOs: 1, 3, 5, and 7.

10. The double-stranded molecule of claim 9, wherein said double-stranded molecule is a single ribonucleotide transcript comprising the sense strand and the antisense strand linked via a single-stranded ribonucleotide sequence.

11. The double-stranded molecule of claim 8, wherein the double-stranded molecule is an oligonucleotide of less than about 100 nucleotides in length.

12. The double-stranded molecule of claim 11, wherein the double-stranded molecule is an oligonucleotide of less than about 75 nucleotides in length.

13. The double-stranded molecule of claim 12, wherein the double-stranded molecule is an oligonucleotide of less than about 50 nucleotides in length.

14. The double-stranded molecule of claim 13, wherein the double-stranded molecule is an oligonucleotide of less than about 25 nucleotides in length.

15. The double-stranded polynucleotide of claim 14, wherein the double stranded molecule is an oligonucleotide of between about 19 and about 25 nucleotides in length.

16. A vector encoding the double-stranded molecule of claim 8.

17. The vector of claim 16, wherein the vector encodes a transcript having a secondary structure and comprises the sense strand and the antisense strand.

18. The vector of claim 17, wherein the transcript further comprises a single-stranded ribonucleotide sequence linking said sense strand and said antisense strand.

19. A vector comprising a polynucleotide comprising a combination of a sense strand nucleic acid and an antisense strand nucleic acid, wherein said sense strand nucleic acid comprises nucleotide sequence of SEQ ID NOs: 54, 57, 60 and 66, and said antisense strand nucleic acid consists of a sequence complementary to the sense strand.

20. The vector of claim 19, wherein said polynucleotide has the general formula

5'-[A]-[B]-[A']-3'

wherein [A] is a nucleotide sequence of SEQ ID NOs: 54, 57, 60 and 66; [B] is a nucleotide sequence consisting of 3 to 23 nucleotides; and [A'] is a nucleotide sequence complementary to [A].

21. A pharmaceutical composition for treating or preventing pancreatic cancer comprising a pharmaceutically effective amount of a small interfering RNA (siRNA) of *PCDH1*, *CDH3*, *GPR107* or *EPHA4* as an active ingredient, and a pharmaceutically acceptable carrier..

22. The pharmaceutical composition of claim 21, wherein the siRNA comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs: 54, 57, 60 and 66 as the target sequence.

23. The composition of claim 22, wherein the siRNA has the general formula

5'-[A]-[B]-[A']-3'

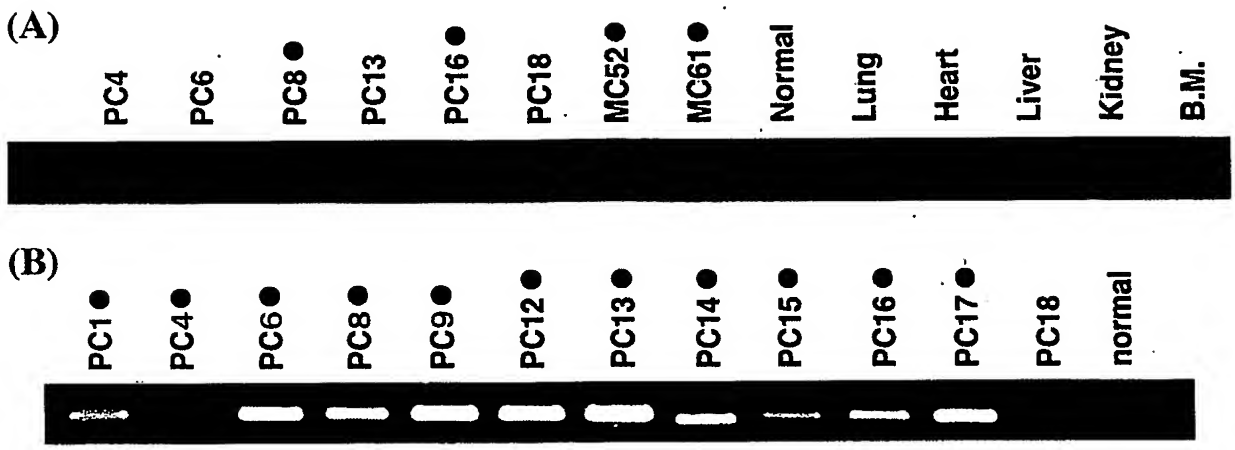
wherein [A] is a ribonucleotide sequence corresponding to a nucleotide sequence of SEQ ID NOs: 54, 57, 60 and 66; [B] is a ribonucleotide sequence consisting of 3 to 23 nucleotides; and [A'] is a ribonucleotide sequence complementary to [A].

****

ABSTRACT

The invention features a method for inhibiting growth of a cancer cell by contacting the cell with a composition of a siRNA of *PCDH1*, *CDH3*, *GPR107* or *EPHA4*. Methods of treating cancer are also within the invention. The invention also features products, including nucleic acid sequences and vectors as well as to compositions comprising them, useful in the provided methods. The invention also provides a method for inhibiting of tumor cell, for example pancreatic cancer cell, particularly pancreatic ductal adenocarcinoma (PDACa).

Fig. 1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Heart
Liver
Kidney
B.M.
(B)
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PC4●
PC6●
PC8●
PC9●
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PC18
normal

Fig. 2

(A) CDH3

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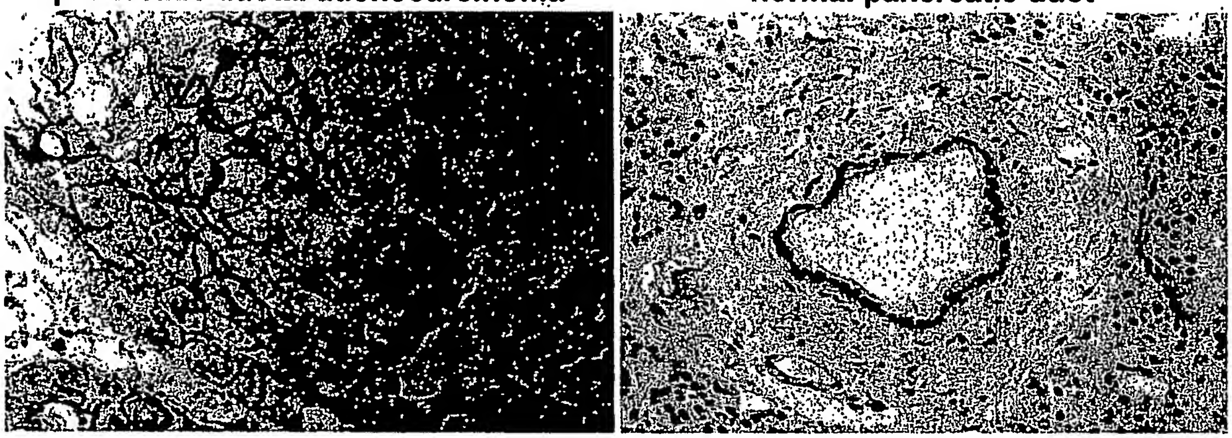


(B) EphA4

pancreatic ductal adenocarcinoma   normal pancreatic duct

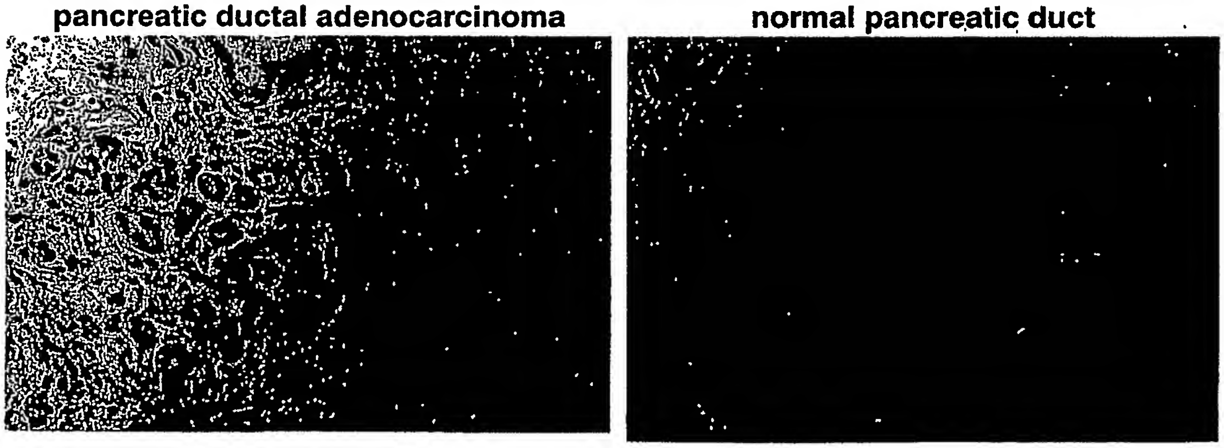
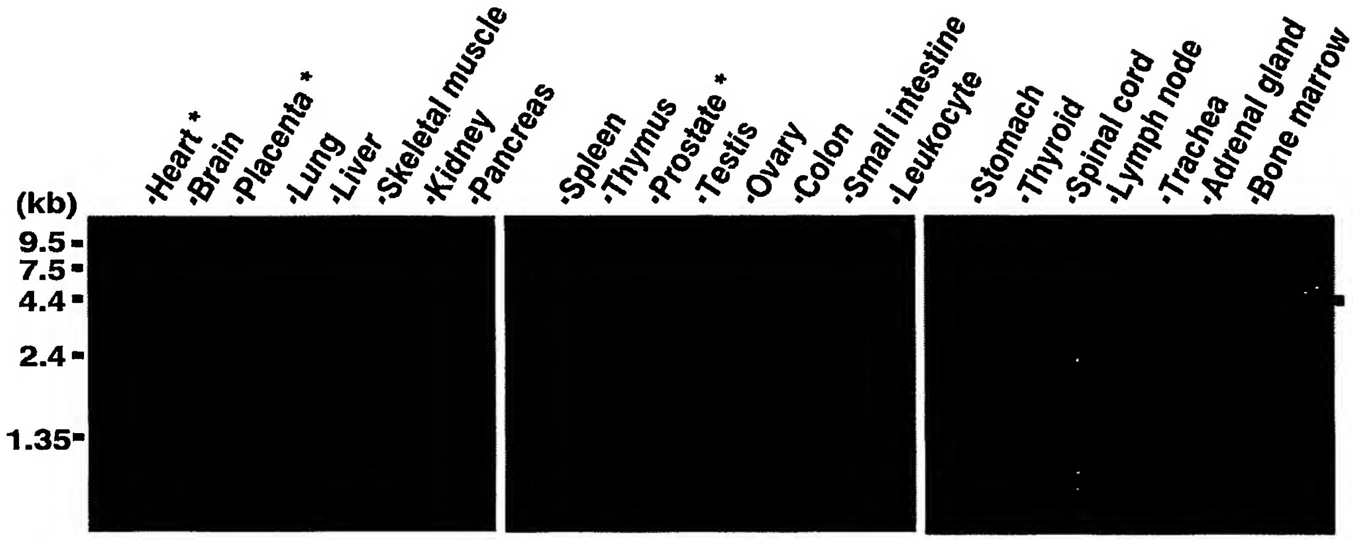


Fig. 3-1

(A) PCDH1



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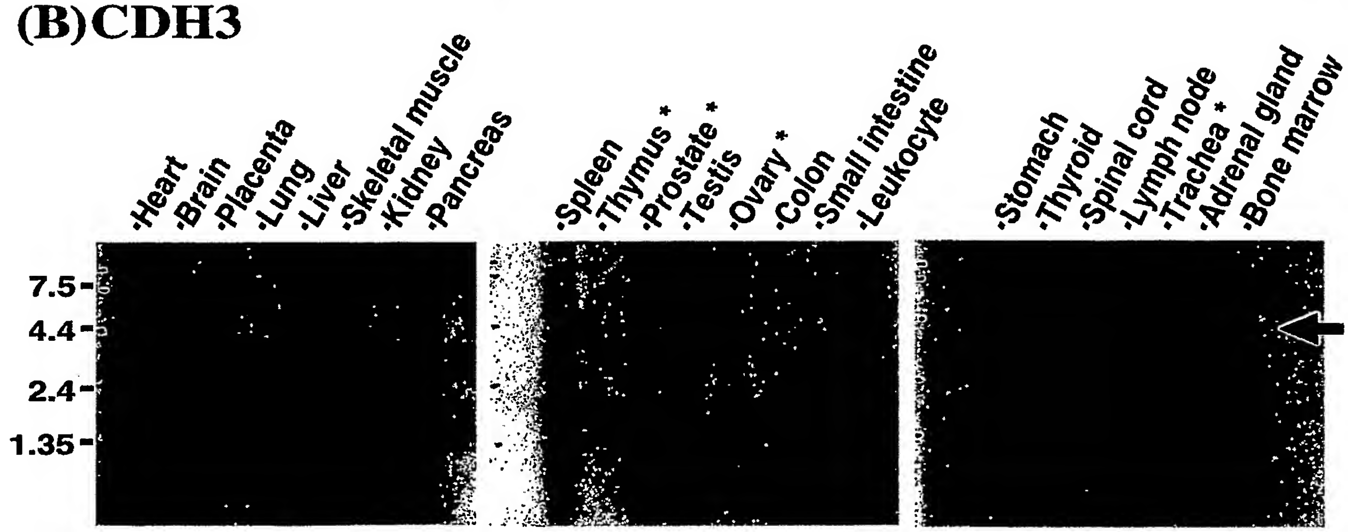
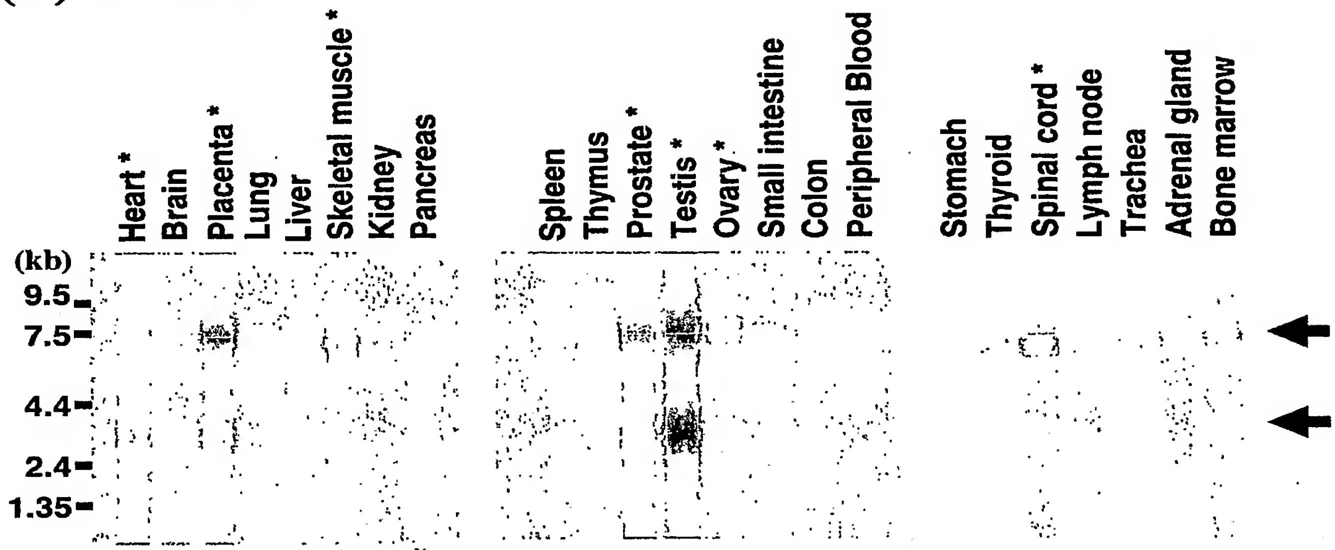
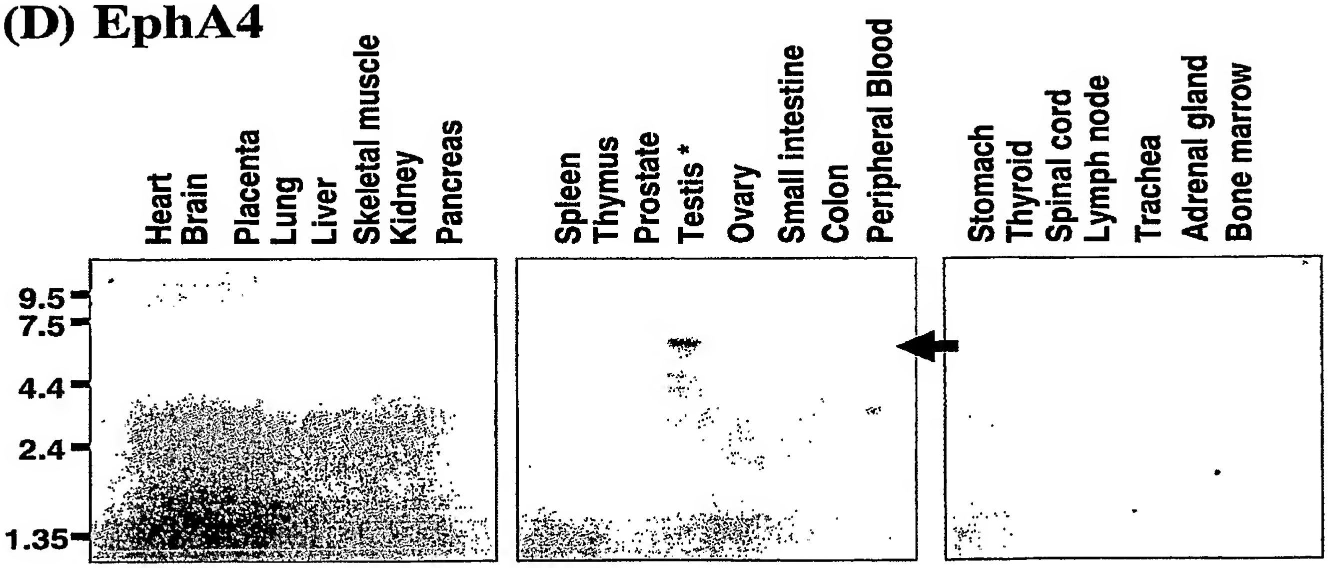


Fig. 3-2

(C) GPR107



(D) EphA4



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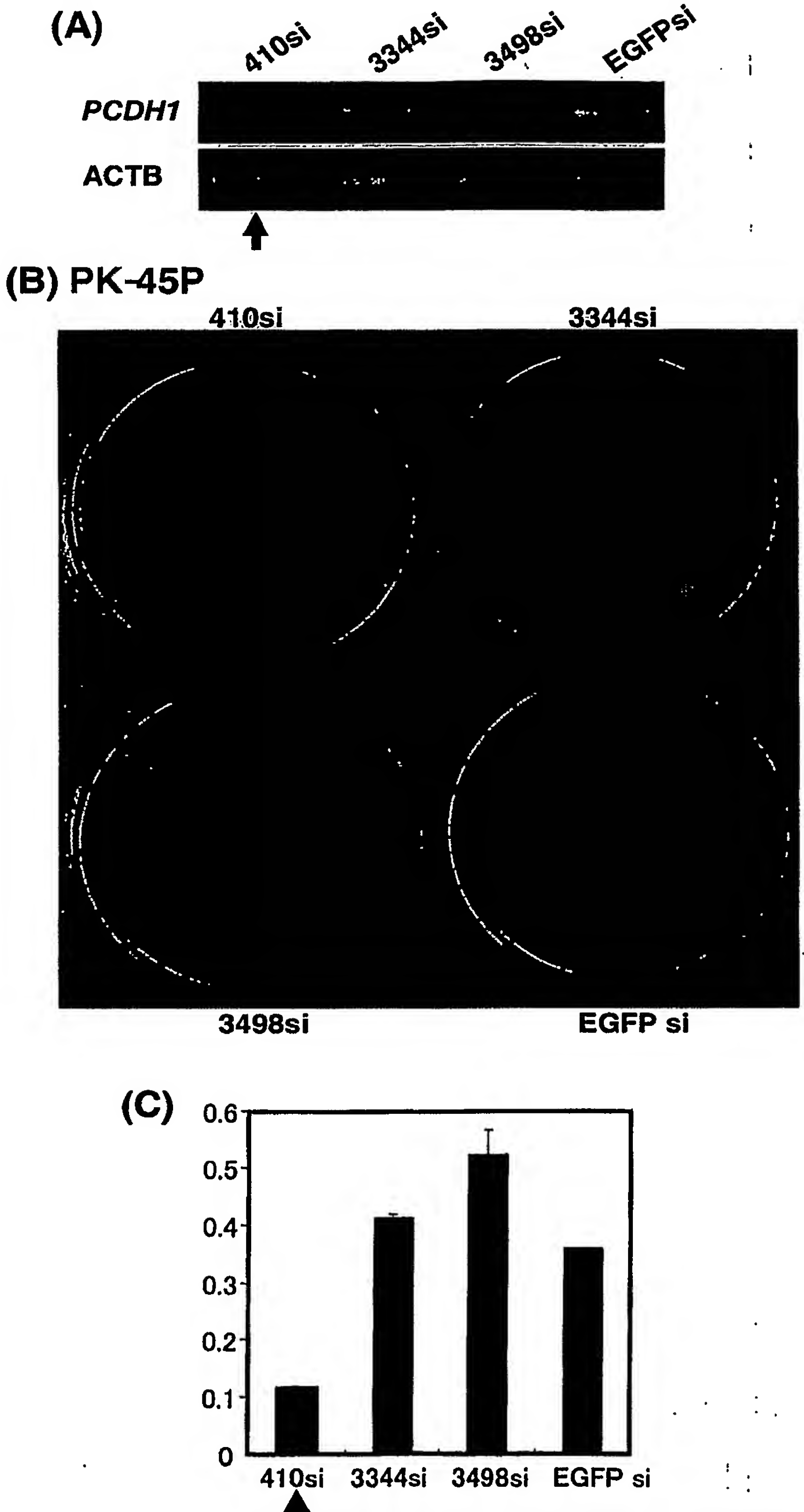
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0
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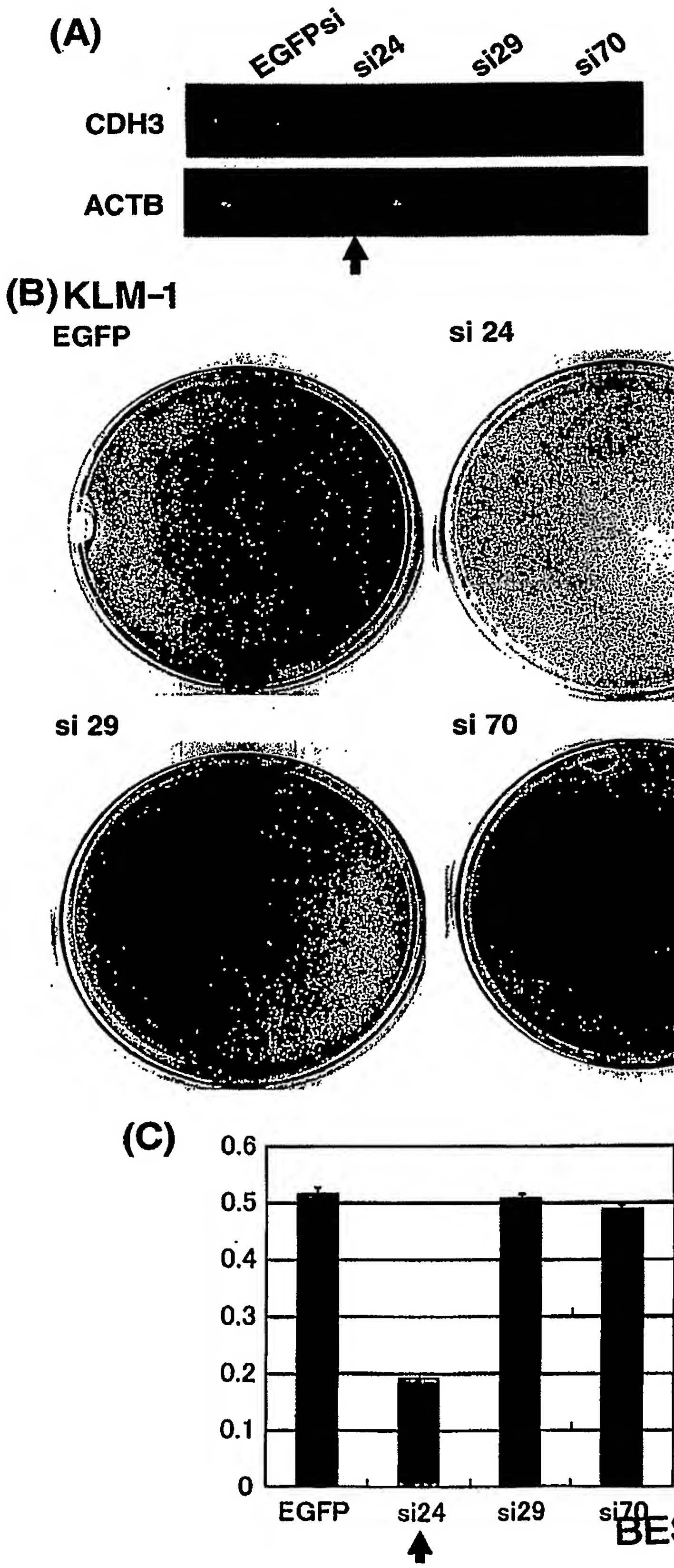
3498si
EGFP si

Fig. 5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si70

Fig. 6

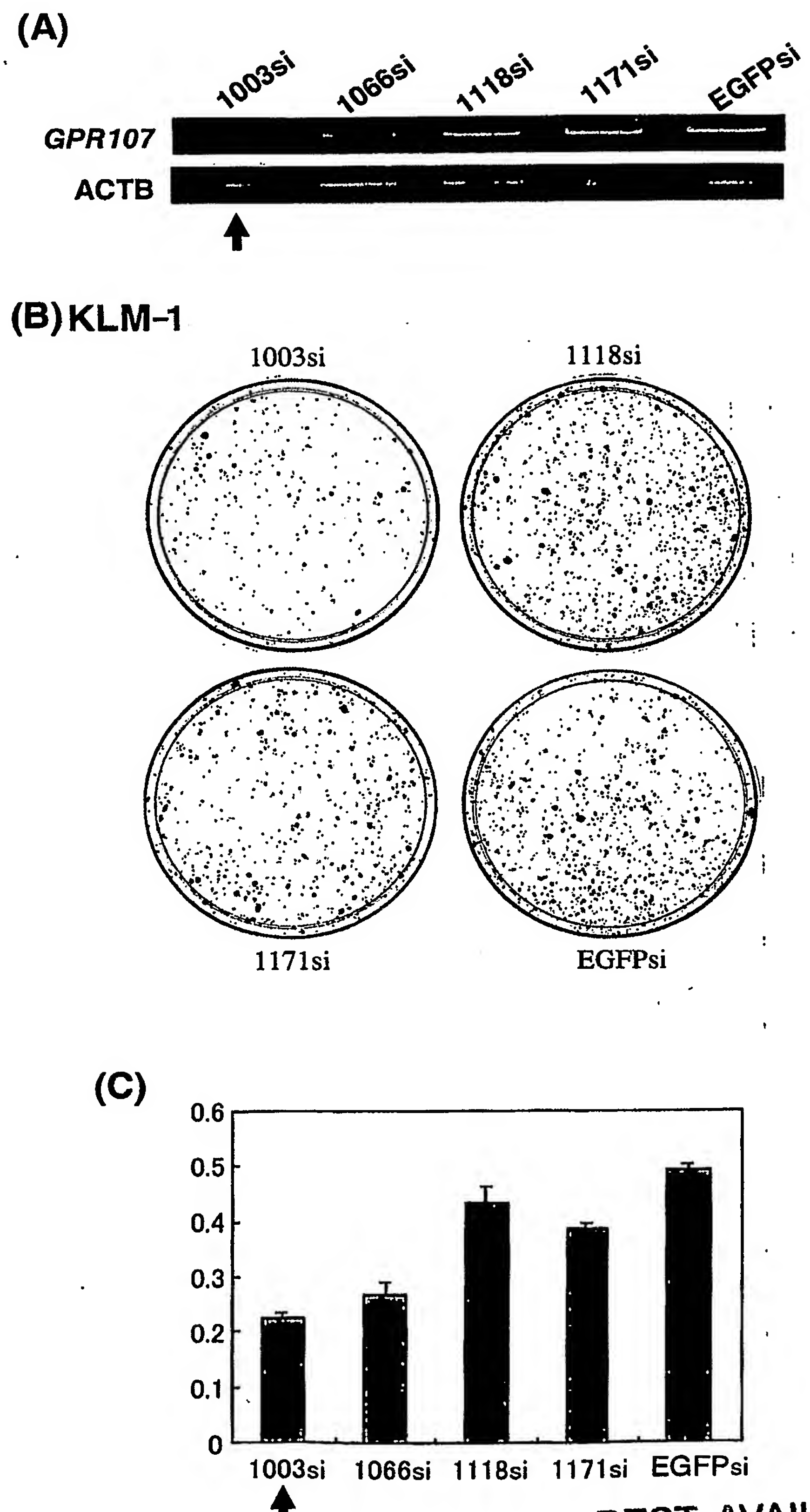
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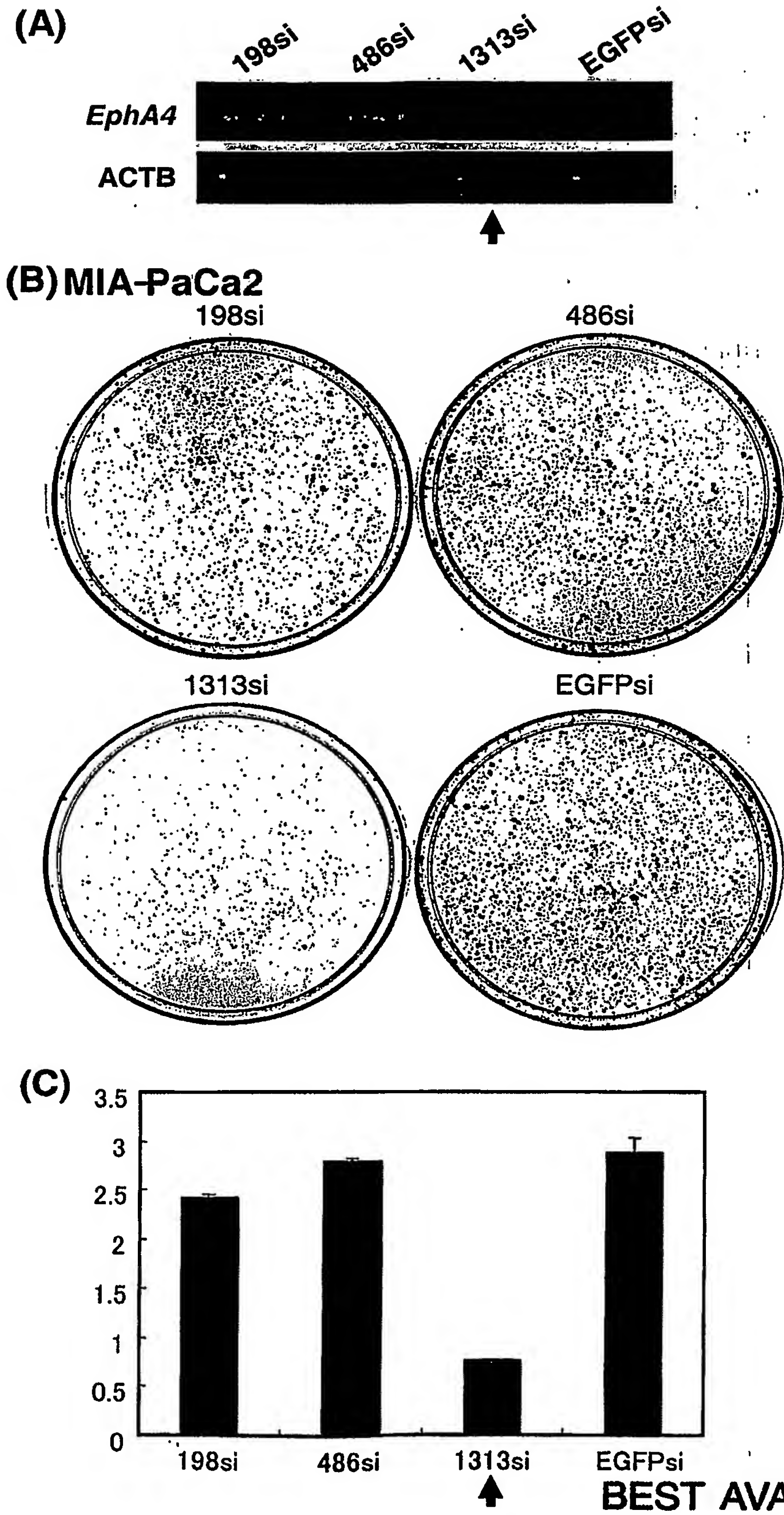
Fig. 8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Asp Phe Val Ile Gln Asn Gly Thr Gly Thr Ile Leu Ser Ser Leu Ser
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Phe Asp Arg Glu Gln Gln Ser Thr Tyr Thr Phe Gln Leu Lys Ala Val
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gat ggt ggc gtc cca cct cgc tca gct tac gtt ggt gtc acc atc aat      2229
Asp Gly Gly Val Pro Pro Arg Ser Ala Tyr Val Gly Val Thr Ile Asn
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gtg ctg gac gag aat gac aac gca ccc tat atc act gcc cct tct aac      2277
Val Leu Asp Glu Asn Asp Asn Ala Pro Tyr Ile Thr Ala Pro Ser Asn
705                 710                 715                 720

acc tct cac aag ctg ctg acc ccc cag aca cgt ctt ggt gag acg gtc      2325
Thr Ser His Lys Leu Leu Thr Pro Gln Thr Arg Leu Gly Glu Thr Val
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agc cag gtg gca gcc gag gac ttt gac tct ggt gtc aat gct gag ctg      2373
Ser Gln Val Ala Ala Glu Asp Phe Asp Ser Gly Val Asn Ala Glu Leu
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atc tac agc att gca ggt ggc aac cct tat gga ctc ttc cag att ggg      2421
Ile Tyr Ser Ile Ala Gly Gly Asn Pro Tyr Gly Leu Phe Gln Ile Gly
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tca cat tca ggt gcc atc acc ctg gag aag gag att gag cgg cgc cac      2469
Ser His Ser Gly Ala Ile Thr Leu Glu Lys Glu Ile Glu Arg Arg His
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cat ggg cta cac cgc ctg gtg gtg aag gtc agt gac cgc ggc aag ccc      2517
His Gly Leu His Arg Leu Val Val Lys Val Ser Asp Arg Gly Lys Pro
785                 790                 795                 800

cca cgc tat ggc aca gcc ttg gtc cat ctt tat gtc aat gag act ctg      2565
Pro Arg Tyr Gly Thr Ala Leu Val His Leu Tyr Val Asn Glu Thr Leu
                805                 810                 815

gcc aac cgc acg ctg ctg gag acc ctc ctg ggc cac agc ctg gac acg      2613
Ala Asn Arg Thr Leu Leu Glu Thr Leu Leu Gly His Ser Leu Asp Thr
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ccg ctg gat att gac att gct ggg gat cca gaa tat gag cgc tcc aag      2661
Pro Leu Asp Ile Asp Ile Ala Gly Asp Pro Glu Tyr Glu Arg Ser Lys
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Gln Arg Gly Asn Ile Leu Phe Gly Val Val Ala Gly Val Val Ala Val
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gcc ttg ctc atc gcc ctg gcg gtt ctt gtg cgc tac tgc aga cag cgg      2757
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gag gcc aaa agt ggt tac cag gct ggt aag aag gag acc aag gac ctg      2805
Glu Ala Lys Ser Gly Tyr Gln Ala Gly Lys Lys Glu Thr Lys Asp Leu
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Tyr Ala Pro Lys Pro Ser Gly Lys Ala Ser Lys Gly Asn Lys Ser Lys
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Gly Lys Lys Ser Lys Ser Pro Lys Pro Val Lys Pro Val Glu Asp Glu
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gat gag gcc ggg ctg cag aag tcc ctc aag ttc aac ctg atg agc gat      2949
Asp Glu Ala Gly Leu Gln Lys Ser Leu Lys Phe Asn Leu Met Ser Asp
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gcc cct ggg gac agt ccc cgc atc cac ctg ccc ctc aac tac cca cca      2997
Ala Pro Gly Asp Ser Pro Arg Ile His Leu Pro Leu Asn Tyr Pro Pro
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Gly Ser Pro Asp Leu Gly Arg His Tyr Arg Ser Asn Ser Pro Leu Pro
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tcc atc cag ctg cag ccc cag tca ccc tca gcc tcc aag aag cac cag      3093
Ser Ile Gln Leu Gln Pro Gln Ser Pro Ser Ala Ser Lys Lys His Gln
            980                 985                 990

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Val Val Gln Asp Leu Pro Pro Ala  Asn Thr Phe Val Gly  Thr Gly Asp
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acc acg  tcc acg ggc tct gag  cag tac tcc gac tac  agc tac cgc       3186
Thr Thr  Ser Thr Gly Ser Glu  Gln Tyr Ser Asp Tyr  Ser Tyr Arg
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Thr Asn  Pro Pro Lys Tyr Pro  Ser Lys Gln Val Gly  Gln Pro Phe
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cag ctc  agc aca ccc cag ccc  cta ccc cac ccc tac  cac gga gcc       3276
Gln Leu  Ser Thr Pro Gln Pro  Leu Pro His Pro Tyr  His Gly Ala
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Ile Trp  Thr Glu Val Trp Glu
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Pro Tyr Leu Arg Val Asp Gly Lys Thr Gly Asp Ile Phe Thr Thr Glu
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Gln Asn Gly Ser Pro Arg Leu Leu Glu Gly Gln Ile Glu Val Gln Asp
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Ile Pro Glu Asn Thr Asn Ile Gly Ser Leu Phe Pro Ile Pro Leu Ala
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Ser Asp Arg Asp Ala Gly Pro Asn Gly Val Ala Ser Tyr Glu Leu Gln
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Gln Glu Glu Lys Gln Pro Gln Leu Ile Val Met Gly Asn Leu Asp Arg
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Glu Arg Trp Asp Ser Tyr Asp Leu Thr Ile Lys Val Gln Asp Gly Gly
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Ser Pro Pro Arg Ala Ser Ser Ala Leu Leu Arg Val Thr Val Leu Asp
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Thr Asn Asp Asn Ala Pro Lys Phe Glu Arg Pro Ser Tyr Glu Ala Glu
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Asn Asp Ser Asp Gln Gly Ala Asn Ala Glu Ile Glu Tyr Thr Phe His
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Gly Leu Ile Thr Val Gln Gly Pro Val Asp Arg Glu Asp Leu Ser Thr
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Ala Arg Ala Gln Val Val Val Thr Val Lys Asp Met Asn Asp Asn Ala
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Pro Thr Ile Glu Ile Arg Gly Ile Gly Leu Val Thr His Gln Asp Gly
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Gln Gly Thr Ala Thr Val Leu Val Asn Val Leu Asp Cys Asn Asp Asn
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Val Leu Asp Glu Asn Asp Asn Ala Pro Tyr Ile Thr Ala Pro Ser Asn
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Thr Ser His Lys Leu Leu Thr Pro Gln Thr Arg Leu Gly Glu Thr Val
725 730 735

Ser Gln Val Ala Ala Glu Asp Phe Asp Ser Gly Val Asn Ala Glu Leu
740 745 750

Ile Tyr Ser Ile Ala Gly Gly Asn Pro Tyr Gly Leu Phe Gln Ile Gly
755 760 765

Ser His Ser Gly Ala Ile Thr Leu Glu Lys Glu Ile Glu Arg Arg His
770 775 780

His Gly Leu His Arg Leu Val Val Lys Val Ser Asp Arg Gly Lys Pro
785 790 795 800

Pro Arg Tyr Gly Thr Ala Leu Val His Leu Tyr Val Asn Glu Thr Leu
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Ala Asn Arg Thr Leu Leu Glu Thr Leu Leu Gly His Ser Leu Asp Thr
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Pro Leu Asp Ile Asp Ile Ala Gly Asp Pro Glu Tyr Glu Arg Ser Lys
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Gln Arg Gly Asn Ile Leu Phe Gly Val Val Ala Gly Val Val Ala Val
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Asp Glu Ala Gly Leu Gln Lys Ser Leu Lys Phe Asn Leu Met Ser Asp
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Ala Pro Gly Asp Ser Pro Arg Ile His Leu Pro Leu Asn Tyr Pro Pro
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Gly Ser Pro Asp Leu Gly Arg His Tyr Arg Ser Asn Ser Pro Leu Pro
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Ser Ile Gln Leu Gln Pro Gln Ser Pro Ser Ala Ser Lys Lys His Gln
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Val Val Gln Asp Leu Pro Pro Ala  Asn Thr Phe Val Gly  Thr Gly Asp
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Leu Gln Val Cys Trp Leu Gln Cys Ala Ala Ser Glu Pro Cys Arg Ala
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Val Phe Arg Glu Ala Glu Val Thr Leu Glu Ala Gly Gly Ala Glu Gln
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aag atc ttc cca tcc aaa cgt atc tta cga aga cac aag aga gat tgg 397
Lys Ile Phe Pro Ser Lys Arg Ile Leu Arg Arg His Lys Arg Asp Trp
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Pro Pro Thr Thr Gly Thr Gly Thr Leu Leu Leu Thr Leu Ile Asp Val
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815 820 825

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Glu Ile Ala Lys Tyr Glu Leu Phe Gly His Ala Val Ser Glu Asn Gly
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Ala Ser Val Glu Asp Pro Met Asn Ile Ser Ile Ile Val Thr Asp Gln
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Thr Ile Ile Pro Thr Pro Met Tyr Arg Pro Arg Pro Ala Asn Pro Asp
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Glu Ile Gly Asn Phe Ile Ile Glu Asn Leu Lys Ala Ala Asn Thr Asp
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Pro Thr Ala Pro Pro Tyr Asp Thr Leu Leu Val Phe Asp Tyr Glu Gly
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Ser Gly Ser Asp Ala Ala Ser Leu Ser Ser Leu Thr Ser Ser Ala Ser
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Ala Glu Pro Gly Leu Gly Arg Val His His Leu Ala Leu Lys Asp Asp
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Val Arg His Lys Val His Leu Asn Thr Phe Gly Phe Phe Lys Asp Gly
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Tyr Met Val Val Asn Val Ser Ser Leu Ser Leu Asn Glu Pro Glu Asp
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Lys Asp Val Thr Ile Gly Phe Ser Leu Asp Arg Thr Lys Asn Asp Gly
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Phe Ser Ser Tyr Leu Asp Glu Asp Val Asn Tyr Cys Ile Leu Lys Lys
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Gln Ser Val Ser Val Thr Leu Leu Ile Leu Asp Ile Ser Arg Ser Glu
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Pro Val Val Trp Ser Ile Arg His Leu Gln Glu Ala Ser Ala Thr Asp
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Thr Tyr Gln Val Cys Asn Val Met Glu Pro Ser Gln Asn Asn Trp Leu
    70                  75                  80

cga act gat tgg atc acc cga gaa ggg gct cag agg gtg tat att gag      342
Arg Thr Asp Trp Ile Thr Arg Glu Gly Ala Gln Arg Val Tyr Ile Glu
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85                  90                  95                  100

att aaa ttc acc ttg agg gac tgc aat agt ctt ccg ggc gtc atg ggg      390
Ile Lys Phe Thr Leu Arg Asp Cys Asn Ser Leu Pro Gly Val Met Gly
                105                 110                 115

act tgc aag gag acg ttt aac ctg tac tac tat gaa tca gac aac gac      438
Thr Cys Lys Glu Thr Phe Asn Leu Tyr Tyr Tyr Glu Ser Asp Asn Asp
            120                 125                 130

aaa gag cgt ttc atc aga gag aac cag ttt gtc aaa att gac acc att      486
Lys Glu Arg Phe Ile Arg Glu Asn Gln Phe Val Lys Ile Asp Thr Ile
        135                 140                 145

gct gct gat gag agc ttc acc caa gtg gac att ggt gac aga atc atg      534
Ala Ala Asp Glu Ser Phe Thr Gln Val Asp Ile Gly Asp Arg Ile Met
    150                 155                 160

aag ctg aac acc gag atc cgg gat gta ggg cca tta agc aaa aag ggg      582
Lys Leu Asn Thr Glu Ile Arg Asp Val Gly Pro Leu Ser Lys Lys Gly
165                 170                 175                 180

ttt tac ctg gct ttt cag gat gtg ggg gcc tgc atc gcc ctg gta tca      630
Phe Tyr Leu Ala Phe Gln Asp Val Gly Ala Cys Ile Ala Leu Val Ser
                185                 190                 195

gtc cgt gtg ttc tat aaa aag tgt cca ctc aca gtc cgc aat ctg gcc      678
Val Arg Val Phe Tyr Lys Lys Cys Pro Leu Thr Val Arg Asn Leu Ala
            200                 205                 210

cag ttt cct gac acc atc aca ggg gct gat acg tct tcc ctg gtg gaa      726
Gln Phe Pro Asp Thr Ile Thr Gly Ala Asp Thr Ser Ser Leu Val Glu
        215                 220                 225

gtt cga ggc tcc tgt gtc aac aac tca gaa gag aaa gat gtg cca aaa      774
Val Arg Gly Ser Cys Val Asn Asn Ser Glu Glu Lys Asp Val Pro Lys
    230                 235                 240

atg tac tgt ggg gca gat ggt gaa tgg ctg gta ccc att ggc aac tgc      822
Met Tyr Cys Gly Ala Asp Gly Glu Trp Leu Val Pro Ile Gly Asn Cys
245                 250                 255                 260

cta tgc aac gct ggg cat gag gag cgg agc gga gaa tgc caa gct tgc      870
Leu Cys Asn Ala Gly His Glu Glu Arg Ser Gly Glu Cys Gln Ala Cys
                265                 270                 275

aaa att gga tat tac aag gct ctc tcc acg gat gcc acc tgt gcc aag      918
Lys Ile Gly Tyr Tyr Lys Ala Leu Ser Thr Asp Ala Thr Cys Ala Lys
            280                 285                 290

tgc cca ccc cac agc tac tct gtc tgg gaa gga gcc acc tcg tgc acc      966
Cys Pro Pro His Ser Tyr Ser Val Trp Glu Gly Ala Thr Ser Cys Thr
        295                 300                 305

tgt gac cga ggc ttt ttc aga gct gac aac gat gct gcc tct atg ccc     1014
Cys Asp Arg Gly Phe Phe Arg Ala Asp Asn Asp Ala Ala Ser Met Pro
    310                 315                 320

tgc acc cgt cca cca tct gct ccc ctg aac ttg att tca aat gtc aac     1062
Cys Thr Arg Pro Pro Ser Ala Pro Leu Asn Leu Ile Ser Asn Val Asn
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325 330 335 340

gag aca tct gtg aac ttg gaa tgg agt agc cct cag aat aca ggt ggc 1110
Glu Thr Ser Val Asn Leu Glu Trp Ser Ser Pro Gln Asn Thr Gly Gly
345 350 355

cgc cag gac att tcc tat aat gtg gta tgc aag aaa tgt gga gct ggt 1158
Arg Gln Asp Ile Ser Tyr Asn Val Val Cys Lys Lys Cys Gly Ala Gly
360 365 370

gac ccc agc aag tgc cga ccc tgt gga agt ggg gtc cac tac acc cca 1206
Asp Pro Ser Lys Cys Arg Pro Cys Gly Ser Gly Val His Tyr Thr Pro
375 380 385

cag cag aat ggc ttg aag acc acc aaa gtc tcc atc act gac ctc cta 1254
Gln Gln Asn Gly Leu Lys Thr Thr Lys Val Ser Ile Thr Asp Leu Leu
390 395 400

gct cat acc aat tac acc ttt gaa atc tgg gct gtg aat gga gtg tcc 1302
Ala His Thr Asn Tyr Thr Phe Glu Ile Trp Ala Val Asn Gly Val Ser
405 410 415 420

aaa tat aac cct aac cca gac caa tca gtt tct gtc act gtg acc acc 1350
Lys Tyr Asn Pro Asn Pro Asp Gln Ser Val Ser Val Thr Val Thr Thr
425 430 435

aac caa gca gca cca tca tcc att gct ttg gtc cag gct aaa gaa gtc 1398
Asn Gln Ala Ala Pro Ser Ser Ile Ala Leu Val Gln Ala Lys Glu Val
440 445 450

aca aga tac agt gtg gca ctg gct tgg ctg gaa cca gat cgg ccc aat 1446
Thr Arg Tyr Ser Val Ala Leu Ala Trp Leu Glu Pro Asp Arg Pro Asn
455 460 465

ggg gta atc ctg gaa tat gaa gtc aag tat tat gag aag gat cag aat 1494
Gly Val Ile Leu Glu Tyr Glu Val Lys Tyr Tyr Glu Lys Asp Gln Asn
470 475 480

gag cga agc tat cgt ata gtt cgg aca gct gcc agg aac aca gat atc 1542
Glu Arg Ser Tyr Arg Ile Val Arg Thr Ala Ala Arg Asn Thr Asp Ile
485 490 495 500

aaa ggc ctg aac cct ctc act tcc tat gtt ttc cac gtg cga gcc agg 1590
Lys Gly Leu Asn Pro Leu Thr Ser Tyr Val Phe His Val Arg Ala Arg
505 510 515

aca gca gct ggc tat gga gac ttc agt gag ccc ttg gag gtt aca acc 1638
Thr Ala Ala Gly Tyr Gly Asp Phe Ser Glu Pro Leu Glu Val Thr Thr
520 525 530

aac aca gtg cct tcc cgg atc att gga gat ggg gct aac tcc aca gtc 1686
Asn Thr Val Pro Ser Arg Ile Ile Gly Asp Gly Ala Asn Ser Thr Val
535 540 545

ctt ctg gtc tct gtc tcg ggc agt gtg gtg ctg gtg gta att ctc att 1734
Leu Leu Val Ser Val Ser Gly Ser Val Val Leu Val Val Ile Leu Ile
550 555 560

gca gct ttt gtc atc agc cgg aga cgg agt aaa tac agt aaa gcc aaa 1782
Ala Ala Phe Val Ile Ser Arg Arg Arg Ser Lys Tyr Ser Lys Ala Lys

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565                 570                 575                 580

caa gaa gcg gat gaa gag aaa cat ttg aat caa ggt gta aga aca tat        1830
Gln Glu Ala Asp Glu Glu Lys His Leu Asn Gln Gly Val Arg Thr Tyr
                585                 590                 595

gtg gac ccc ttt acg tac gaa gat ccc aac caa gca gtg cga gag ttt        1878
Val Asp Pro Phe Thr Tyr Glu Asp Pro Asn Gln Ala Val Arg Glu Phe
            600                 605                 610

gcc aaa gaa att gac gca tcc tgc att aag att gaa aaa gtt ata gga        1926
Ala Lys Glu Ile Asp Ala Ser Cys Ile Lys Ile Glu Lys Val Ile Gly
        615                 620                 625

gtt ggt gaa ttt ggt gag gta tgc agt ggg cgt ctc aaa gtg cct ggc        1974
Val Gly Glu Phe Gly Glu Val Cys Ser Gly Arg Leu Lys Val Pro Gly
    630                 635                 640

aag aga gag atc tgt gtg gct atc aag act ctg aaa gct ggt tat aca        2022
Lys Arg Glu Ile Cys Val Ala Ile Lys Thr Leu Lys Ala Gly Tyr Thr
645                 650                 655                 660

gac aaa cag agg aga gac ttc ctg agt gag gcc agc atc atg gga cag        2070
Asp Lys Gln Arg Arg Asp Phe Leu Ser Glu Ala Ser Ile Met Gly Gln
                665                 670                 675

ttt gac cat ccg aac atc att cac ttg gaa ggc gtg gtc act aaa tgt        2118
Phe Asp His Pro Asn Ile Ile His Leu Glu Gly Val Val Thr Lys Cys
            680                 685                 690

aaa cca gta atg atc ata aca gag tac atg gag aat ggc tcc ttg gat        2166
Lys Pro Val Met Ile Ile Thr Glu Tyr Met Glu Asn Gly Ser Leu Asp
        695                 700                 705

gca ttc ctc agg aaa aat gat ggc aga ttt aca gtc att cag ctg gtg        2214
Ala Phe Leu Arg Lys Asn Asp Gly Arg Phe Thr Val Ile Gln Leu Val
    710                 715                 720

ggc atg ctt cgt ggc att ggg tct ggg atg aag tat tta tct gat atg        2262
Gly Met Leu Arg Gly Ile Gly Ser Gly Met Lys Tyr Leu Ser Asp Met
725                 730                 735                 740

agc tat gtg cat cgt gat ctg gcc gca cgg aac atc ctg gtg aac agc        2310
Ser Tyr Val His Arg Asp Leu Ala Ala Arg Asn Ile Leu Val Asn Ser
                745                 750                 755

aac ttg gtc tgc aaa gtg tct gat ttt ggc atg tcc cga gtg ctt gag        2358
Asn Leu Val Cys Lys Val Ser Asp Phe Gly Met Ser Arg Val Leu Glu
            760                 765                 770

gat gat ccg gaa gca gct tac acc acc agg ggt ggc aag att cct atc        2406
Asp Asp Pro Glu Ala Ala Tyr Thr Thr Arg Gly Gly Lys Ile Pro Ile
        775                 780                 785

cgg tgg act gcg cca gaa gca att gcc tat cgt aaa ttc aca tca gca        2454
Arg Trp Thr Ala Pro Glu Ala Ile Ala Tyr Arg Lys Phe Thr Ser Ala
    790                 795                 800

agt gat gta tgg agc tat gga atc gtt atg tgg gaa gtg atg tcg tac        2502
Ser Asp Val Trp Ser Tyr Gly Ile Val Met Trp Glu Val Met Ser Tyr
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805                 810                 815                 820

ggg gag agg ccc tat tgg gat atg tcc aat caa gat gtg att aaa gcc      2550
Gly Glu Arg Pro Tyr Trp Asp Met Ser Asn Gln Asp Val Ile Lys Ala
                825                 830                 835

att gag gaa ggc tat cgg tta ccc cct cca atg gac tgc ccc att gcg      2598
Ile Glu Glu Gly Tyr Arg Leu Pro Pro Pro Met Asp Cys Pro Ile Ala
            840                 845                 850

ctc cac cag ctg atg cta gac tgc tgg cag aag gag agg agc gac agg      2646
Leu His Gln Leu Met Leu Asp Cys Trp Gln Lys Glu Arg Ser Asp Arg
        855                 860                 865

cct aaa ttt ggg cag att gtc aac atg ttg gac aaa ctc atc cgc aac      2694
Pro Lys Phe Gly Gln Ile Val Asn Met Leu Asp Lys Leu Ile Arg Asn
    870                 875                 880

ccc aac agc ttg aag agg aca ggg acg gag agc tcc aga cct aac act      2742
Pro Asn Ser Leu Lys Arg Thr Gly Thr Glu Ser Ser Arg Pro Asn Thr
885                 890                 895                 900

gcc ttg ttg gat cca agc tcc cct gaa ttc tct gct gtg gta tca gtg      2790
Ala Leu Leu Asp Pro Ser Ser Pro Glu Phe Ser Ala Val Val Ser Val
                905                 910                 915

ggc gat tgg ctc cag gcc att aaa atg gac cgg tat aag gat aac ttc      2838
Gly Asp Trp Leu Gln Ala Ile Lys Met Asp Arg Tyr Lys Asp Asn Phe
            920                 925                 930

aca gct gct ggt tat acc aca cta gag gct gtg gtg cac gtg aac cag      2886
Thr Ala Ala Gly Tyr Thr Thr Leu Glu Ala Val Val His Val Asn Gln
        935                 940                 945

gag gac ctg gca aga att ggt atc aca gcc atc acg cac cag aat aag      2934
Glu Asp Leu Ala Arg Ile Gly Ile Thr Ala Ile Thr His Gln Asn Lys
    950                 955                 960

att ttg agc agt gtc cag gca atg cga acc caa atg cag cag atg cac      2982
Ile Leu Ser Ser Val Gln Ala Met Arg Thr Gln Met Gln Gln Met His
965                 970                 975                 980

ggc aga atg gtt ccc gtc tga gccagtactg aataaactca aaactcttga        3033
Gly Arg Met Val Pro Val
                985

aattagttta cctcatccat gcactttaat tgaagaactg cacttttttt acttcgtctt    3093
cgccctctga aattaaagaa atgaaaaaaa aaaacaatat ctgcagcgtt gcttggtgca    3153
cagattgctg aaactgtggg gcttacagaa atgactgccg gtcatttgaa tgagacctgg    3213
aacaaatcgt ttctcagaag tacttttctg ttcatcacca gtctgtaaaa tacatgtacc    3273
tatagaaata gaacactgcc tctgagtttt gatgctgtat ttgctgccag acactgagct    3333
tctgagacat ccctgattct ctctccattt ggaattacaa ccattgtatt ttgtttgtgg    3393
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Cys Asp Ala Val Thr Gly Ser Arg Val Tyr Pro Ala Asn Glu Val Thr
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Leu Leu Asp Ser Arg Ser Val Gln Gly Glu Leu Gly Trp Ile Ala Ser
        35   40   45

Pro Leu Glu Gly Gly Trp Glu Glu Val Ser Ile Met Asp Glu Lys Asn
    50   55   60

Thr Pro Ile Arg Thr Tyr Gln Val Cys Asn Val Met Glu Pro Ser Gln
65   70   75   80

Asn Asn Trp Leu Arg Thr Asp Trp Ile Thr Arg Glu Gly Ala Gln Arg
                85   90   95

Val Tyr Ile Glu Ile Lys Phe Thr Leu Arg Asp Cys Asn Ser Leu Pro
            100   105   110

Gly Val Met Gly Thr Cys Lys Glu Thr Phe Asn Leu Tyr Tyr Tyr Glu
        115   120   125

Ser Asp Asn Asp Lys Glu Arg Phe Ile Arg Glu Asn Gln Phe Val Lys
    130   135   140

Ile Asp Thr Ile Ala Ala Asp Glu Ser Phe Thr Gln Val Asp Ile Gly
145   150   155   160

Asp Arg Ile Met Lys Leu Asn Thr Glu Ile Arg Asp Val Gly Pro Leu
                165   170   175

Ser Lys Lys Gly Phe Tyr Leu Ala Phe Gln Asp Val Gly Ala Cys Ile
            180   185   190

Ala Leu Val Ser Val Arg Val Phe Tyr Lys Lys Cys Pro Leu Thr Val
        195   200   205

Arg Asn Leu Ala Gln Phe Pro Asp Thr Ile Thr Gly Ala Asp Thr Ser
    210   215   220

Ser Leu Val Glu Val Arg Gly Ser Cys Val Asn Asn Ser Glu Glu Lys
225   230   235   240

Asp Val Pro Lys Met Tyr Cys Gly Ala Asp Gly Glu Trp Leu Val Pro
                245   250   255

Ile Gly Asn Cys Leu Cys Asn Ala Gly His Glu Glu Arg Ser Gly Glu
            260   265   270

Cys Gln Ala Cys Lys Ile Gly Tyr Tyr Lys Ala Leu Ser Thr Asp Ala
        275   280   285

Thr Cys Ala Lys Cys Pro Pro His Ser Tyr Ser Val Trp Glu Gly Ala
    290   295   300

Thr Ser Cys Thr Cys Asp Arg Gly Phe Phe Arg Ala Asp Asn Asp Ala

305 310 315 320

Ala Ser Met Pro Cys Thr Arg Pro Pro Ser Ala Pro Leu Asn Leu Ile
325 330 335

Ser Asn Val Asn Glu Thr Ser Val Asn Leu Glu Trp Ser Ser Pro Gln
340 345 350

Asn Thr Gly Gly Arg Gln Asp Ile Ser Tyr Asn Val Val Cys Lys Lys
355 360 365

Cys Gly Ala Gly Asp Pro Ser Lys Cys Arg Pro Cys Gly Ser Gly Val
370 375 380

His Tyr Thr Pro Gln Gln Asn Gly Leu Lys Thr Thr Lys Val Ser Ile
385 390 395 400

Thr Asp Leu Leu Ala His Thr Asn Tyr Thr Phe Glu Ile Trp Ala Val
405 410 415

Asn Gly Val Ser Lys Tyr Asn Pro Asn Pro Asp Gln Ser Val Ser Val
420 425 430

Thr Val Thr Thr Asn Gln Ala Ala Pro Ser Ser Ile Ala Leu Val Gln
435 440 445

Ala Lys Glu Val Thr Arg Tyr Ser Val Ala Leu Ala Trp Leu Glu Pro
450 455 460

Asp Arg Pro Asn Gly Val Ile Leu Glu Tyr Glu Val Lys Tyr Tyr Glu
465 470 475 480

Lys Asp Gln Asn Glu Arg Ser Tyr Arg Ile Val Arg Thr Ala Ala Arg
485 490 495

Asn Thr Asp Ile Lys Gly Leu Asn Pro Leu Thr Ser Tyr Val Phe His
500 505 510

Val Arg Ala Arg Thr Ala Ala Gly Tyr Gly Asp Phe Ser Glu Pro Leu
515 520 525

Glu Val Thr Thr Asn Thr Val Pro Ser Arg Ile Ile Gly Asp Gly Ala
530 535 540

Asn Ser Thr Val Leu Leu Val Ser Val Ser Gly Ser Val Val Leu Val
545 550 555 560

Val Ile Leu Ile Ala Ala Phe Val Ile Ser Arg Arg Arg Ser Lys Tyr
565 570 575

Ser Lys Ala Lys Gln Glu Ala Asp Glu Glu Lys His Leu Asn Gln Gly
580 585 590

Val Arg Thr Tyr Val Asp Pro Phe Thr Tyr Glu Asp Pro Asn Gln Ala
595 600 605

Val Arg Glu Phe Ala Lys Glu Ile Asp Ala Ser Cys Ile Lys Ile Glu
610 615 620

Lys Val Ile Gly Val Gly Glu Phe Gly Glu Val Cys Ser Gly Arg Leu

625 630 635 640

Lys Val Pro Gly Lys Arg Glu Ile Cys Val Ala Ile Lys Thr Leu Lys
645 650 655

Ala Gly Tyr Thr Asp Lys Gln Arg Arg Asp Phe Leu Ser Glu Ala Ser
660 665 670

Ile Met Gly Gln Phe Asp His Pro Asn Ile Ile His Leu Glu Gly Val
675 680 685

Val Thr Lys Cys Lys Pro Val Met Ile Ile Thr Glu Tyr Met Glu Asn
690 695 700

Gly Ser Leu Asp Ala Phe Leu Arg Lys Asn Asp Gly Arg Phe Thr Val
705 710 715 720

Ile Gln Leu Val Gly Met Leu Arg Gly Ile Gly Ser Gly Met Lys Tyr
725 730 735

Leu Ser Asp Met Ser Tyr Val His Arg Asp Leu Ala Ala Arg Asn Ile
740 745 750

Leu Val Asn Ser Asn Leu Val Cys Lys Val Ser Asp Phe Gly Met Ser
755 760 765

Arg Val Leu Glu Asp Asp Pro Glu Ala Ala Tyr Thr Thr Arg Gly Gly
770 775 780

Lys Ile Pro Ile Arg Trp Thr Ala Pro Glu Ala Ile Ala Tyr Arg Lys
785 790 795 800

Phe Thr Ser Ala Ser Asp Val Trp Ser Tyr Gly Ile Val Met Trp Glu
805 810 815

Val Met Ser Tyr Gly Glu Arg Pro Tyr Trp Asp Met Ser Asn Gln Asp
820 825 830

Val Ile Lys Ala Ile Glu Glu Gly Tyr Arg Leu Pro Pro Pro Met Asp
835 840 845

Cys Pro Ile Ala Leu His Gln Leu Met Leu Asp Cys Trp Gln Lys Glu
850 855 860

Arg Ser Asp Arg Pro Lys Phe Gly Gln Ile Val Asn Met Leu Asp Lys
865 870 875 880

Leu Ile Arg Asn Pro Asn Ser Leu Lys Arg Thr Gly Thr Glu Ser Ser
885 890 895

Arg Pro Asn Thr Ala Leu Leu Asp Pro Ser Ser Pro Glu Phe Ser Ala
900 905 910

Val Val Ser Val Gly Asp Trp Leu Gln Ala Ile Lys Met Asp Arg Tyr
915 920 925

Lys Asp Asn Phe Thr Ala Ala Gly Tyr Thr Thr Leu Glu Ala Val Val
930 935 940

His Val Asn Gln Glu Asp Leu Ala Arg Ile Gly Ile Thr Ala Ile Thr

945 950 955 960

His Gln Asn Lys Ile Leu Ser Ser Val Gln Ala Met Arg Thr Gln Met
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Gln Gln Met His Gly Arg Met Val Pro Val
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